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(54) **Title:** PHOSPHOLIPASES, NUCLEIC ACIDS ENCODING THEM AND METHODS FOR MAKING AND USING THEM

(57) **Abstract:** The invention provides novel polypeptides having phospholipase activity, including, e.g., phospholipase A, B, C and D activity, patatin activity, lipid acyl hydrolase (LAH) activity, nucleic acids encoding them and antibodies that bind to them. Industrial methods, e.g., oil degumming, and products comprising use of these phospholipase are also provided.

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PHOSPHOLIPASES, NUCLEIC ACIDS ENCODING THEM AND METHODS FOR MAKING AND USING THEM

FIELD OF THE INVENTION

This invention relates generally to phospholipase enzymes, polynucleotides encoding the enzymes, methods of making and using these polynucleotides and polypeptides. In particular, the invention provides novel polypeptides having phospholipase activity, nucleic acids encoding them and antibodies that bind to them. Industrial methods and products comprising use of these phospholipases are also provided.

BACKGROUND

Phospholipases are enzymes that hydrolyze the ester bonds of phospholipids. Corresponding to their importance in the metabolism of phospholipids, these enzymes are widespread among prokaryotes and eukaryotes. The phospholipases affect the metabolism, construction and reorganization of biological membranes and are involved in signal cascades. Several types of phospholipases are known which differ in their specificity according to the position of the bond attacked in the phospholipid molecule. Phospholipase A1 (PLA1) removes the 1-position fatty acid to produce free fatty acid and 1-lyso-2-acylphospholipid. Phospholipase A2 (PLA2) removes the 2-position fatty acid to produce free fatty acid and 1-acyl-2-lysophospholipid. PLA1 and PLA2 enzymes can be intra- or extra-cellular, membrane-bound or soluble. Intracellular PLA2 is found in almost every mammalian cell. Phospholipase C (PLC) removes the phosphate moiety to produce 1,2 diacylglycerol and phospho base. Phospholipase D (PLD) produces 1,2-diacylglycerophosphate and base group. PLC and PLD are important in cell function and signaling. PLD had been the dominant phospholipase in biocatalysis (see, e.g., Godfrey, T. and West S. (1996) Industrial enzymology, 299-300, Stockton Press, New York). Patatins are another type of phospholipase, thought to work as a PLA (see for example, Hirschberg HJ, et al., (2001), Eur J Biochem 268(19):5037-44).

Common oilseeds, such as soybeans, rapeseed, sunflower seeds, sesame and peanuts are used as sources of oils and feedstock. In the oil extraction process, the seeds are mechanically and thermally treated. The oil is separated and divided from the meal by a solvent. Using distillation, the solvent is then separated from the oil and recovered. The oil is "degummed" and refined. The solvent content in the meal can be evaporated by thermal treatment in a "desolventizer toaster," followed by meal drying and cooling. After a solvent had been separated by distillation, the produced raw oil is processed into edible oil, using

special degumming procedures and physical refining. It can also be utilized as feedstock for the production of fatty acids and methyl ester. The meal can be used for animal rations.

Degumming is the first step in vegetable oil refining and it is designed to remove contaminating phosphatides that are extracted with the oil but interfere with the subsequent oil processing. These phosphatides are soluble in the vegetable oil only in an anhydrous form and can be precipitated and removed if they are simply hydrated. Hydration is usually accomplished by mixing a small proportion of water continuously with substantially dry oil. Typically, the amount of water is 75% of the phosphatides content, which is typically 1 to 1.5 %. The temperature is not highly critical, although separation of the hydrated gums is better if the viscosity of the oil is reduced at 50^0C to 80^0C.

Many methods for oil degumming are currently used. The process of oil degumming can be enzymatically assisted by using phospholipase enzymes. Phospholipases A1 and A2 have been used for oil degumming in various commercial processes, e.g., "ENZYMAX™ degumming" (Lurgi Life Science Technologies GmbH, Germany). Phospholipase C (PLC) also has been considered for oil degumming because the phosphate moiety generated by its action on phospholipids is very water soluble and easy to remove and the diglyceride would stay with the oil and reduce losses; see e.g., Godfrey, T. and West S. (1996) Industrial Enzymology, pp.299-300, Stockton Press, New York; Dahlke (1998) "An enzymatic process for the physical refining of seed oils," Chem. Eng. Technol. 21:278-281; Clausen (2001) "Enzymatic oil degumming by a novel microbial phospholipase," Eur. J. Lipid Sci. Technol. 103:333-340.

High phosphatide oils such as soy, canola and sunflower are processed differently than other oils such as palm. Unlike the steam or "physical refining" process for low phosphatide oils, these high phosphorous oils require special chemical and mechanical treatments to remove the phosphorous-containing phospholipids. These oils are typically refined chemically in a process that entails neutralizing the free fatty acids to form soap and an insoluble gum fraction. The neutralization process is highly effective in removing free fatty acids and phospholipids but this process also results in significant yield losses and sacrifices in quality. In some cases, the high phosphatide crude oil is degummed in a step preceding caustic neutralization. This is the case for soy oil utilized for lecithin wherein the oil is first water or acid degummed.

Phytosterols (plant sterols) are members of the "triterpene" family of natural products, which includes more than 100 different phytosterols and more than 4000 other types of triterpenes. In general, phytosterols are thought to stabilize plant membranes, with

an increase in the sterol/phospholipid ration leading to membrane rigidification. Chemically, phytosterols closely resemble cholesterol in structure. The major phytosterols are β-sitosterol, campesterol and stigmasterol. Others include stigmastanol (β-sitostanol), sitostanol, desmosterol, chalinasterol, poriferasterol, clionasterol and brassicasterol.

Plant sterols are important agricultural products for health and nutritional industries. They are useful emulsifiers for cosmetic manufacturers and supply the majority of steroidal intermediates and precursors for the production of hormone pharmaceuticals. The saturated analogs of phytosterols and their esters have been suggested as effective cholesterol-lowering agents with cardiologic health benefits. Plant sterols reduce serum cholesterol levels by inhibiting cholesterol absorption in the intestinal lumen and have immunomodulating properties at extremely low concentrations, including enhanced cellular response of T lymphocytes and cytotoxic ability of natural killer cells against a cancer cell line. In addition, their therapeutic effect has been demonstrated in clinical studies for treatment of pulmonary tuberculosis, rheumatoid arthritis, management of HIV-infested patients and inhibition of immune stress in marathon runners.

Plant sterol esters, also referred to as phytosterol esters, were approved as GRAS (Generally Recognized As Safe) by the US Food and Drug Administration (FDA) for use in margarines and spreads in 1999. In September 2000, the FDA also issued an interim rule that allows health-claims labeling of foods containing phytosterol ester. Consequently enrichment of foods with phytosterol esters is highly desired for consumer acceptance.

SUMMARY OF THE INVENTION

The invention provides isolated or recombinant nucleic acids comprising a nucleic acid sequence having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to an exemplary nucleic acid of the invention, e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69,

SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105 over a region of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900, 1950, 2000, 2050, 2100, 2200, 2250, 2300, 2350, 2400, 2450, 2500, or more residues, encodes at least one polypeptide having a phospholipase, e.g., a phospholipase A, C or D activity, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection.

The invention provides isolated or recombinant nucleic acids comprising a nucleic acid sequence having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:1 over a region of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850 more consecutive residues, wherein the nucleic acids encode at least one polypeptide having a phospholipase, e.g., a phospholipase A, B, C or D activity and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection.

The invention provides isolated or recombinant nucleic acids comprising a nucleic acid sequence having at least 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:3 over a region of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850 more consecutive residues, wherein the nucleic acids encode at least one polypeptide having a phospholipase, e.g., a phospholipase A, B, C or D activity and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection.

The invention provides isolated or recombinant nucleic acids comprising a nucleic acid sequence having at least 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:5 over a region of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850 more consecutive residues, wherein the nucleic acids encode at least one polypeptide having a phospholipase, e.g., a phospholipase A, B, C or D activity and the

sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection.

The invention provides isolated or recombinant nucleic acids comprising a nucleic acid sequence having at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:7 over a region of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850 more consecutive residues, wherein the nucleic acids encode at least one polypeptide having a phospholipase, e.g., a phospholipase A, B, C or D activity and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection.

In alternative aspects, the isolated or recombinant nucleic acid encodes a polypeptide comprising a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. In one aspect these polypeptides have a phospholipase, e.g., a phospholipase A, B, C or D activity.

In one aspect, the sequence comparison algorithm is a BLAST algorithm, such as a BLAST version 2.2.2 algorithm. In one aspect, the filtering setting is set to blastall -p blastp -d "nr pataa" -F F and all other options are set to default.

In one aspect, the phospholipase activity comprises catalyzing hydrolysis of a glycerolphosphate ester linkage (i.e., cleavage of glycerolphosphate ester linkages). The phospholipase activity can comprise catalyzing hydrolysis of an ester linkage in a phospholipid in a vegetable oil. The vegetable oil phospholipid can comprise an oilseed phospholipid. The phospholipase activity can comprise a phospholipase C (PLC) activity, a phospholipase A (PLA) activity, such as a phospholipase A1 or phospholipase A2 activity, a phospholipase D (PLD) activity, such as a phospholipase D1 or a phospholipase D2 activity, or patatin activity. The phospholipase activity can comprise hydrolysis of a glycoprotein, e.g., as a glycoprotein found in a potato tuber. The phospholipase activity can comprise a patatin enzymatic activity. The phospholipase activity can comprise a lipid acyl hydrolase (LAH) activity.

In one aspect, the isolated or recombinant nucleic acid encodes a polypeptide having a phospholipase activity which is thermostable. The polypeptide can retain a phospholipase activity under conditions comprising a temperature range of between about 37°C to about 95°C; between about 55°C to about 85°C, between about 70°C to about 95°C,

or, between about 90°C to about 95°C. In another aspect, the isolated or recombinant nucleic acid encodes a polypeptide having a phospholipase activity which is thermotolerant. The polypeptide can retain a phospholipase activity after exposure to a temperature in the range from greater than 37°C to about 95°C or anywhere in the range from greater than 55°C to about 85°C. In one aspect, the polypeptide retains a phospholipase activity after exposure to a temperature in the range from greater than 90°C to about 95°C at pH 4.5.

The polypeptide can retain a phospholipase activity under conditions comprising about pH 7, pH 6.5, pH 6.0, pH 5.5, pH 5, or pH 4.5. The polypeptide can retain a phospholipase activity under conditions comprising a temperature range of between about 40°C to about 70°C.

In one aspect, the isolated or recombinant nucleic acid comprises a sequence that hybridizes under stringent conditions to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7, wherein the nucleic acid encodes a polypeptide having a phospholipase activity. The nucleic acid can at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850 or residues in length or the full length of the gene or transcript, with or without a signal sequence, as described herein. The stringent conditions can be highly stringent, moderately stringent or of low stringency, as described herein. The stringent conditions can include a wash step, e.g., a wash step comprising a wash in 0.2X SSC at a temperature of about 65°C for about 15 minutes.

The invention provides a nucleic acid probe for identifying a nucleic acid encoding a polypeptide with a phospholipase, e.g., a phospholipase, activity, wherein the probe comprises at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, or more, consecutive bases of a sequence of the invention, e.g., a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7, and the probe identifies the nucleic acid by binding or hybridization. The probe can comprise an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 consecutive bases of a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and/or SEQ ID NO:7.

The invention provides a nucleic acid probe for identifying a nucleic acid encoding a polypeptide with a phospholipase, e.g., a phospholipase activity, wherein the probe comprises a nucleic acid of the invention, e.g., a nucleic acid having at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%,

84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and/or SEQ ID NO:7, or a subsequence thereof, over a region of at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850 or more consecutive residues, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection.

The invention provides an amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having a phospholipase activity, wherein the primer pair is capable of amplifying a nucleic acid comprising a sequence of the invention, or fragments or subsequences thereof. One or each member of the amplification primer sequence pair can comprise an oligonucleotide comprising at least about 10 to 50 consecutive bases of the sequence, or about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 consecutive bases of the sequence.

The invention provides amplification primer pairs, wherein the primer pair comprises a first member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues of a nucleic acid of the invention, and a second member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues of the complementary strand of the first member.

The invention provides phospholipases generated by amplification, e.g., polymerase chain reaction (PCR), using an amplification primer pair of the invention. The invention provides methods of making a phospholipase by amplification, e.g., polymerase chain reaction (PCR), using an amplification primer pair of the invention. In one aspect, the amplification primer pair amplifies a nucleic acid from a library, e.g., a gene library, such as an environmental library.

The invention provides methods of amplifying a nucleic acid encoding a polypeptide having a phospholipase activity comprising amplification of a template nucleic acid with an amplification primer sequence pair capable of amplifying a nucleic acid sequence of the invention, or fragments or subsequences thereof. The amplification primer pair can be an amplification primer pair of the invention.

The invention provides expression cassettes comprising a nucleic acid of the invention or a subsequence thereof. In one aspect, the expression cassette can comprise the nucleic acid that is operably linked to a promoter. The promoter can be a viral, bacterial, mammalian or plant promoter. In one aspect, the plant promoter can be a potato, rice, corn, wheat, tobacco or barley promoter. The promoter can be a constitutive promoter. The

constitutive promoter can comprise CaMV35S. In another aspect, the promoter can be an inducible promoter. In one aspect, the promoter can be a tissue-specific promoter or an environmentally regulated or a developmentally regulated promoter. Thus, the promoter can be, e.g., a seed-specific, a leaf-specific, a root-specific, a stem-specific or an abscission-induced promoter. In one aspect, the expression cassette can further comprise a plant or plant virus expression vector.

The invention provides cloning vehicles comprising an expression cassette (e.g., a vector) of the invention or a nucleic acid of the invention. The cloning vehicle can be a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome. The viral vector can comprise an adenovirus vector, a retroviral vector or an adeno-associated viral vector. The cloning vehicle can comprise a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC).

The invention provides transformed cell comprising a nucleic acid of the invention or an expression cassette (e.g., a vector) of the invention, or a cloning vehicle of the invention. In one aspect, the transformed cell can be a bacterial cell, a mammalian cell, a fungal cell, a yeast cell, an insect cell or a plant cell. In one aspect, the plant cell can be a potato, wheat, rice, corn, tobacco or barley cell.

The invention provides transgenic non-human animals comprising a nucleic acid of the invention or an expression cassette (e.g., a vector) of the invention. In one aspect, the animal is a mouse.

The invention provides transgenic plants comprising a nucleic acid of the invention or an expression cassette (e.g., a vector) of the invention. The transgenic plant can be a corn plant, a potato plant, a tomato plant, a wheat plant, an oilseed plant, a rapeseed plant, a soybean plant, a rice plant, a barley plant or a tobacco plant. The invention provides transgenic seeds comprising a nucleic acid of the invention or an expression cassette (e.g., a vector) of the invention. The transgenic seed can be a corn seed, a wheat kernel, an oilseed, a rapeseed (a canola plant), a soybean seed, a palm kernel, a sunflower seed, a sesame seed, a peanut or a tobacco plant seed.

The invention provides an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a nucleic acid of the invention. The invention provides methods of inhibiting the translation of a phospholipase message in a cell comprising administering to the cell or expressing in the cell

an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a nucleic acid of the invention.

The invention provides an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a nucleic acid of the invention. The invention provides methods of inhibiting the translation of a phospholipase message in a cell comprising administering to the cell or expressing in the cell an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a nucleic acid of the invention. The antisense oligonucleotide can be between about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, about 60 to 100, about 70 to 110, or about 80 to 120 bases in length.

The invention provides methods of inhibiting the translation of a phospholipase, e.g., a phospholipase, message in a cell comprising administering to the cell or expressing in the cell an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a nucleic acid of the invention. The invention provides double-stranded inhibitory RNA (RNAi) molecules comprising a subsequence of a sequence of the invention. In one aspect, the RNAi is about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more duplex nucleotides in length. The invention provides methods of inhibiting the expression of a phospholipase, e.g., a phospholipase, in a cell comprising administering to the cell or expressing in the cell a double-stranded inhibitory RNA (iRNA), wherein the RNA comprises a subsequence of a sequence of the invention.

The invention provides an isolated or recombinant polypeptide comprising an amino acid sequence having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to an exemplary polypeptide or peptide of the invention over a region of at least about 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350 or more residues, or over the full length of the polypeptide, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection. Exemplary polypeptide or peptide sequences of the invention include SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8. In one aspect, the invention provides an isolated or recombinant polypeptide comprising an amino acid sequence having at least about 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:2. In one aspect, the invention

provides an isolated or recombinant polypeptide comprising an amino acid sequence having at least about 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:4. In one aspect, the invention provides an isolated or recombinant polypeptide comprising an amino acid sequence having at least about 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:6. In one aspect, the invention provides an isolated or recombinant polypeptide comprising an amino acid sequence having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to SEQ ID NO:8. The invention provides isolated or recombinant polypeptides encoded by a nucleic acid of the invention. In alternative aspects, the polypeptide can have a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8. The polypeptide can have a phospholipase activity, e.g., a phospholipase A, B, C or D activity.

The invention provides isolated or recombinant polypeptides comprising a polypeptide of the invention lacking a signal sequence. In one aspect, the polypeptide lacking a signal sequence has at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to residues 30 to 287 of SEQ ID NO:2, an amino acid sequence having at least 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to residues 25 to 283 of SEQ ID NO:4, an amino acid sequence having at least 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to residues 26 to 280 of SEQ ID NO:6, or, an amino acid sequence having at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to residues 40 to 330 of SEQ ID NO:8. The sequence identities can be determined by analysis with a sequence comparison algorithm or by visual inspection.

Another aspect of the invention provides an isolated or recombinant polypeptide or peptide including at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 or more consecutive bases of a polypeptide or peptide sequence of the

invention, sequences substantially identical thereto, and the sequences complementary thereto. The peptide can be, e.g., an immunogenic fragment, a motif (e.g., a binding site) or an active site.

In one aspect, the isolated or recombinant polypeptide of the invention (with or without a signal sequence) has a phospholipase activity. In one aspect, the phospholipase activity comprises catalyzing hydrolysis of a glycerolphosphate ester linkage (i.e., cleavage of glycerolphosphate ester linkages). The phospholipase activity can comprise catalyzing hydrolysis of an ester linkage in a phospholipid in a vegetable oil. The vegetable oil phospholipid can comprise an oilseed phospholipid. The phospholipase activity can comprise a phospholipase C (PLC) activity, a phospholipase A (PLA) activity, such as a phospholipase A1 or phospholipase A2 activity, a phospholipase D (PLD) activity, such as a phospholipase D1 or a phospholipase D2 activity. The phospholipase activity can comprise hydrolysis of a glycoprotein, e.g., as a glycoprotein found in a potato tuber. The phospholipase activity can comprise a patatin enzymatic activity. The phospholipase activity can comprise a lipid acyl hydrolase (LAH) activity.

In one aspect, the phospholipase activity is thermostable. The polypeptide can retain a phospholipase activity under conditions comprising a temperature range of between about 37°C to about 95°C, between about 55°C to about 85°C, between about 70°C to about 95°C, or between about 90°C to about 95°C. In another aspect, the phospholipase activity can be thermotolerant. The polypeptide can retain a phospholipase activity after exposure to a temperature in the range from greater than 37°C to about 95°C, or in the range from greater than 55°C to about 85°C. In one aspect, the polypeptide can retain a phospholipase activity after exposure to a temperature in the range from greater than 90°C to about 95°C at pH 4.5.

In one aspect, the polypeptide can retain a phospholipase activity under conditions comprising about pH 6.5, pH 6, pH 5.5, pH 5, pH 4.5 or pH 4. In another aspect, the polypeptide can retain a phospholipase activity under conditions comprising about pH 7, pH 7.5 pH 8.0, pH 8.5, pH 9, pH 9.5, pH 10, pH 10.5 or pH 11.

In one aspect, the isolated or recombinant polypeptide can comprise the polypeptide of the invention that lacks a signal sequence. In one aspect, the isolated or recombinant polypeptide can comprise the polypeptide of the invention comprising a heterologous signal sequence, such as a heterologous phospholipase or non-phospholipase signal sequence.

The invention provides isolated or recombinant peptides comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or more sequence identity to

residues 1 to 29 of SEQ ID NO:2, at least 95%, 96%, 97%, 98%, 99%, or more sequence identity to residues 1 to 24 of SEQ ID NO:4, at least 95%, 96%, 97%, 98%, 99%, or more sequence identity to residues 1 to 25 of SEQ ID NO:6, or at least 95%, 96%, 97%, 98%, 99%, or more sequence identity to residues 1 to 39 of SEQ ID NO:8, and to other signal sequences as set forth in the SEQ ID listing, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection. These peptides can act as signal sequences on its endogenous phospholipase, on another phospholipase, or a heterologous protein (a non-phospholipase enzyme or other protein). In one aspect, the invention provides chimeric proteins comprising a first domain comprising a signal sequence of the invention and at least a second domain. The protein can be a fusion protein. The second domain can comprise an enzyme. The enzyme can be a phospholipase.

The invention provides chimeric polypeptides comprising at least a first domain comprising signal peptide (SP) of the invention or a catalytic domain (CD), or active site, of a phospholipase of the invention and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP) or catalytic domain (CD). In one aspect, the heterologous polypeptide or peptide is not a phospholipase. The heterologous polypeptide or peptide can be amino terminal to, carboxy terminal to or on both ends of the signal peptide (SP) or catalytic domain (CD).

The invention provides isolated or recombinant nucleic acids encoding a chimeric polypeptide, wherein the chimeric polypeptide comprises at least a first domain comprising signal peptide (SP) or a catalytic domain (CD), or active site, of a polypeptide of the invention, and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP) or catalytic domain (CD).

In one aspect, the phospholipase activity comprises a specific activity at about 37°C in the range from about 100 to about 1000 units per milligram of protein. In another aspect, the phospholipase activity comprises a specific activity from about 500 to about 750 units per milligram of protein. Alternatively, the phospholipase activity comprises a specific activity at 37°C in the range from about 500 to about 1200 units per milligram of protein. In one aspect, the phospholipase activity comprises a specific activity at 37°C in the range from about 750 to about 1000 units per milligram of protein. In another aspect, the thermotolerance comprises retention of at least half of the specific activity of the phospholipase at 37°C after being heated to the elevated temperature. Alternatively, the

thermotolerance can comprise retention of specific activity at 37°C in the range from about 500 to about 1200 units per milligram of protein after being heated to the elevated temperature.

The invention provides the isolated or recombinant polypeptide of the invention, wherein the polypeptide comprises at least one glycosylation site. In one aspect, glycosylation can be an N-linked glycosylation. In one aspect, the polypeptide can be glycosylated after being expressed in a *P. pastoris* or a *S. pombe*.

The invention provides protein preparations comprising a polypeptide of the invention, wherein the protein preparation comprises a liquid, a solid or a gel.

The invention provides heterodimers comprising a polypeptide of the invention and a second protein or domain. The second member of the heterodimer can be a different phospholipase, a different enzyme or another protein. In one aspect, the second domain can be a polypeptide and the heterodimer can be a fusion protein. In one aspect, the second domain can be an epitope or a tag. In one aspect, the invention provides homodimers comprising a polypeptide of the invention.

The invention provides immobilized polypeptides having a phospholipase activity, wherein the polypeptide comprises a polypeptide of the invention, a polypeptide encoded by a nucleic acid of the invention, or a polypeptide comprising a polypeptide of the invention and a second domain. In one aspect, the polypeptide can be immobilized on a cell, a metal, a resin, a polymer, a ceramic, a glass, a microelectrode, a graphitic particle, a bead, a gel, a plate, an array or a capillary tube.

The invention provides arrays comprising an immobilized polypeptide, wherein the polypeptide is a phospholipase of the invention or is a polypeptide encoded by a nucleic acid of the invention. The invention provides arrays comprising an immobilized nucleic acid of the invention. The invention provides an array comprising an immobilized antibody of the invention.

The invention provides isolated or recombinant antibodies that specifically bind to a polypeptide of the invention or to a polypeptide encoded by a nucleic acid of the invention. The antibody can be a monoclonal or a polyclonal antibody. The invention provides hybridomas comprising an antibody of the invention.

The invention provides methods of isolating or identifying a polypeptide with a phospholipase activity comprising the steps of: (a) providing an antibody of the invention; (b) providing a sample comprising polypeptides; and, (c) contacting the sample of step (b) with the antibody of step (a) under conditions wherein the antibody can specifically bind to

the polypeptide, thereby isolating or identifying a phospholipase. The invention provides methods of making an anti-phospholipase antibody comprising administering to a non-human animal a nucleic acid of the invention, or a polypeptide of the invention, in an amount sufficient to generate a humoral immune response, thereby making an anti-phospholipase antibody.

The invention provides methods of producing a recombinant polypeptide comprising the steps of: (a) providing a nucleic acid of the invention operably linked to a promoter; and, (b) expressing the nucleic acid of step (a) under conditions that allow expression of the polypeptide, thereby producing a recombinant polypeptide. The nucleic acid can comprise a sequence having at least 85% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, having at least 80% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues, having at least 80% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues, or having at least 70% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection. The nucleic acid can comprise a nucleic acid that hybridizes under stringent conditions to a nucleic acid as set forth in SEQ ID NO:1, or a subsequence thereof; a sequence as set forth in SEQ ID NO:3, or a subsequence thereof; a sequence as set forth in SEQ ID NO:5, or a subsequence thereof; or, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof. The method can further comprise transforming a host cell with the nucleic acid of step (a) followed by expressing the nucleic acid of step (a), thereby producing a recombinant polypeptide in a transformed cell. The method can further comprise inserting into a host non-human animal the nucleic acid of step (a) followed by expressing the nucleic acid of step (a), thereby producing a recombinant polypeptide in the host non-human animal.

The invention provides methods for identifying a polypeptide having a phospholipase activity comprising the following steps: (a) providing a polypeptide of the invention or a polypeptide encoded by a nucleic acid of the invention, or a fragment or variant thereof, (b) providing a phospholipase substrate; and, (c) contacting the polypeptide or a fragment or variant thereof of step (a) with the substrate of step (b) and detecting an increase in the amount of substrate or a decrease in the amount of reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product detects a polypeptide having a phospholipase activity. In alternative aspects, the nucleic acid comprises a sequence having at least 85% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, having at least 80% sequence identity to SEQ ID NO:3 over a

region of at least about 100 residues, having at least 80% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues, or having at least 70% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection. In alternative aspects the nucleic acid hybridizes under stringent conditions a sequence as set forth in SEQ ID NO:1, or a subsequence thereof; a sequence as set forth in SEQ ID NO:3, or a subsequence thereof; a sequence as set forth in SEQ ID NO:5, or a subsequence thereof; or, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof.

The invention provides methods for identifying a phospholipase substrate comprising the following steps: (a) providing a polypeptide of the invention or a polypeptide encoded by a nucleic acid of the invention; (b) providing a test substrate; and, (c) contacting the polypeptide of step (a) with the test substrate of step (b) and detecting an increase in the amount of substrate or a decrease in the amount of reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product identifies the test substrate as a phospholipase substrate. In alternative aspects, the nucleic acid can have at least 85% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, at least 80% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues, at least 80% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues, or, at least 70% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection. In alternative aspects, the nucleic acid hybridizes under stringent conditions to a sequence as set forth in SEQ ID NO:1, or a subsequence thereof; a sequence as set forth in SEQ ID NO:3, or a subsequence thereof; a sequence as set forth in SEQ ID NO:5, or a subsequence thereof; or, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof.

The invention provides methods of determining whether a compound specifically binds to a phospholipase comprising the following steps: (a) expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide, wherein the nucleic acid and vector comprise a nucleic acid or vector of the invention; or, providing a polypeptide of the invention (b) contacting the polypeptide with the test compound; and, (c) determining whether the test compound specifically binds to the polypeptide, thereby determining that the compound specifically binds to the phospholipase. In alternative aspects, the nucleic acid sequence has at least 85% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues,

at least 80% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues, least 80% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues, or, at least 70% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection. In alternative aspects, the nucleic acid hybridizes under stringent conditions to a sequence as set forth in SEQ ID NO:1, or a subsequence thereof; a sequence as set forth in SEQ ID NO:3, or a subsequence thereof; a sequence as set forth in SEQ ID NO:5, or a subsequence thereof; or, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof.

The invention provides methods for identifying a modulator of a phospholipase activity comprising the following steps: (a) providing a polypeptide of the invention or a polypeptide encoded by a nucleic acid of the invention; (b) providing a test compound; (c) contacting the polypeptide of step (a) with the test compound of step (b); and, measuring an activity of the phospholipase, wherein a change in the phospholipase activity measured in the presence of the test compound compared to the activity in the absence of the test compound provides a determination that the test compound modulates the phospholipase activity. In alternative aspects, the nucleic acid can have at least 85% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues, at least 80% sequence identity to SEQ ID NO:3 over a region of at least about 100 residues, at least 80% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues, or, at least 70% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection. In alternative aspects, the nucleic acid can hybridize under stringent conditions to a nucleic acid sequence selected from the group consisting of a sequence as set forth in SEQ ID NO:1, or a subsequence thereof; a sequence as set forth in SEQ ID NO:3, or a subsequence thereof; a sequence as set forth in SEQ ID NO:5, or a subsequence thereof; and, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof.

In one aspect, the phospholipase activity is measured by providing a phospholipase substrate and detecting an increase in the amount of the substrate or a decrease in the amount of a reaction product. The decrease in the amount of the substrate or the increase in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an activator of phospholipase activity. The increase in the amount of the substrate or the decrease in the amount of the reaction product with the test compound as

compared to the amount of substrate or reaction product without the test compound identifies the test compound as an inhibitor of phospholipase activity.

The invention provides computer systems comprising a processor and a data storage device wherein said data storage device has stored thereon a polypeptide sequence of the invention or a nucleic acid sequence of the invention.

In one aspect, the computer system can further comprise a sequence comparison algorithm and a data storage device having at least one reference sequence stored thereon. The sequence comparison algorithm can comprise a computer program that indicates polymorphisms. The computer system can further comprising an identifier that identifies one or more features in said sequence.

The invention provides computer readable mediums having stored thereon a sequence comprising a polypeptide sequence of the invention or a nucleic acid sequence of the invention.

The invention provides methods for identifying a feature in a sequence comprising the steps of: (a) reading the sequence using a computer program which identifies one or more features in a sequence, wherein the sequence comprises a polypeptide sequence of the invention or a nucleic acid sequence of the invention; and, (b) identifying one or more features in the sequence with the computer program.

The invention provides methods for comparing a first sequence to a second sequence comprising the steps of: (a) reading the first sequence and the second sequence through use of a computer program which compares sequences, wherein the first sequence comprises a polypeptide sequence of the invention or a nucleic acid sequence of the invention; and, (b) determining differences between the first sequence and the second sequence with the computer program. In one aspect, the step of determining differences between the first sequence and the second sequence further comprises the step of identifying polymorphisms. In one aspect, the method further comprises an identifier (and use of the identifier) that identifies one or more features in a sequence. In one aspect, the method comprises reading the first sequence using a computer program and identifying one or more features in the sequence.

The invention provides methods for isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample comprising the steps of: (a) providing an amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide with a phospholipase activity, wherein the primer pair is capable of amplifying a nucleic acid of the invention (e.g., SEQ ID NO:1, or a subsequence

thereof; SEQ ID NO:3, or a subsequence thereof; SEQ ID NO:5, or a subsequence thereof; or SEQ ID NO:7, or a subsequence thereof, etc.); (b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair; and, (c) combining the nucleic acid of step (b) with the amplification primer pair of step (a) and amplifying nucleic acid from the environmental sample, thereby isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample. In one aspect, each member of the amplification primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 consecutive bases of a nucleic acid sequence of the invention. In one aspect, the amplification primer sequence pair is an amplification pair of the invention.

The invention provides methods for isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample comprising the steps of: (a) providing a polynucleotide probe comprising a nucleic acid sequence of the invention, or a subsequence thereof; (b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe of step (a); (c) combining the isolated nucleic acid or the treated environmental sample of step (b) with the polynucleotide probe of step (a); and, (d) isolating a nucleic acid that specifically hybridizes with the polynucleotide probe of step (a), thereby isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from the environmental sample. In alternative aspects, the environmental sample comprises a water sample, a liquid sample, a soil sample, an air sample or a biological sample. In alternative aspects, the biological sample is derived from a bacterial cell, a protozoan cell, an insect cell, a yeast cell, a plant cell, a fungal cell or a mammalian cell.

The invention provides methods of generating a variant of a nucleic acid encoding a phospholipase comprising the steps of: (a) providing a template nucleic acid comprising a nucleic acid of the invention; (b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid.

In one aspect, the method further comprises expressing the variant nucleic acid to generate a variant phospholipase polypeptide. In alternative aspects, the modifications, additions or deletions are introduced by error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette

mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSM), synthetic ligation reassembly (SLR) and/or a combination thereof. In alternative aspects, the modifications, additions or deletions are introduced by a method selected from the group consisting of recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and/or a combination thereof.

In one aspect, the method is iteratively repeated until a phospholipase having an altered or different activity or an altered or different stability from that of a phospholipase encoded by the template nucleic acid is produced. In one aspect, the altered or different activity is a phospholipase activity under an acidic condition, wherein the phospholipase encoded by the template nucleic acid is not active under the acidic condition. In one aspect, the altered or different activity is a phospholipase activity under a high temperature, wherein the phospholipase encoded by the template nucleic acid is not active under the high temperature. In one aspect, the method is iteratively repeated until a phospholipase coding sequence having an altered codon usage from that of the template nucleic acid is produced. The method can be iteratively repeated until a phospholipase gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced.

The invention provides methods for modifying codons in a nucleic acid encoding a phospholipase to increase its expression in a host cell, the method comprising (a) providing a nucleic acid of the invention encoding a phospholipase; and, (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

The invention provides methods for modifying codons in a nucleic acid encoding a phospholipase, the method comprising (a) providing a nucleic acid of the invention encoding a phospholipase; and, (b) identifying a codon in the nucleic acid of step

(a) and replacing it with a different codon encoding the same amino acid as the replaced codon, thereby modifying codons in a nucleic acid encoding a phospholipase.

The invention provides methods for modifying codons in a nucleic acid encoding a phospholipase to increase its expression in a host cell, the method comprising (a) providing a nucleic acid of the invention encoding a phospholipase; and, (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

The invention provides methods for modifying a codon in a nucleic acid encoding a phospholipase to decrease its expression in a host cell, the method comprising (a) providing a nucleic acid of the invention encoding a phospholipase; and, (b) identifying at least one preferred codon in the nucleic acid of step (a) and replacing it with a non-preferred or less preferred codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in a host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to decrease its expression in a host cell. In alternative aspects, the host cell is a bacterial cell, a fungal cell, an insect cell, a yeast cell, a plant cell or a mammalian cell.

The invention provides methods for producing a library of nucleic acids encoding a plurality of modified phospholipase active sites or substrate binding sites, wherein the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site the method comprising: (a) providing a first nucleic acid encoding a first active site or first substrate binding site, wherein the first nucleic acid sequence comprises a nucleic acid of the invention; (b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and, (c) using the set of mutagenic oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of modified phospholipase active sites or substrate binding sites. In alternative aspects, the method comprises mutagenizing the first nucleic acid of step (a) by a

method comprising an optimized directed evolution system, gene site-saturation mutagenesis (GSSM), and synthetic ligation reassembly (SLR). The method can further comprise mutagenizing the first nucleic acid of step (a) or variants by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSM), synthetic ligation reassembly (SLR) and a combination thereof. The method can further comprise mutagenizing the first nucleic acid of step (a) or variants by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

The invention provides methods for making a small molecule comprising the steps of: (a) providing a plurality of biosynthetic enzymes capable of synthesizing or modifying a small molecule, wherein one of the enzymes comprises a phospholipase enzyme encoded by a nucleic acid of the invention; (b) providing a substrate for at least one of the enzymes of step (a); and, (c) reacting the substrate of step (b) with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series of biocatalytic reactions.

The invention provides methods for modifying a small molecule comprising the steps: (a) providing a phospholipase enzyme encoded by a nucleic acid of the invention; (b) providing a small molecule; and, (c) reacting the enzyme of step (a) with the small molecule of step (b) under conditions that facilitate an enzymatic reaction catalyzed by the phospholipase enzyme, thereby modifying a small molecule by a phospholipase enzymatic reaction. In one aspect, the method comprises providing a plurality of small molecule substrates for the enzyme of step (a), thereby generating a library of modified small molecules produced by at least one enzymatic reaction catalyzed by the phospholipase enzyme. In one aspect, the method further comprises a plurality of additional enzymes under conditions that facilitate a plurality of biocatalytic reactions by the enzymes to form a library of modified small molecules produced by the plurality of enzymatic reactions. In one aspect, the method further comprises the step of testing the library to determine if a particular

modified small molecule that exhibits a desired activity is present within the library. The step of testing the library can further comprises the steps of systematically eliminating all but one of the biocatalytic reactions used to produce a portion of the plurality of the modified small molecules within the library by testing the portion of the modified small molecule for the presence or absence of the particular modified small molecule with a desired activity, and identifying at least one specific biocatalytic reaction that produces the particular modified small molecule of desired activity.

The invention provides methods for determining a functional fragment of a phospholipase enzyme comprising the steps of: (a) providing a phospholipase enzyme comprising an amino acid sequence of the invention; and, (b) deleting a plurality of amino acid residues from the sequence of step (a) and testing the remaining subsequence for a phospholipase activity, thereby determining a functional fragment of a phospholipase enzyme. In one aspect, the phospholipase activity is measured by providing a phospholipase substrate and detecting an increase in the amount of the substrate or a decrease in the amount of a reaction product. In one aspect, a decrease in the amount of an enzyme substrate or an increase in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an activator of phospholipase activity.

The invention provides methods for cleaving a glycerolphosphate ester linkage comprising the following steps: (a) providing a polypeptide having a phospholipase activity, wherein the polypeptide comprises an amino acid sequence of the invention, or the polypeptide is encoded by a nucleic acid of the invention; (b) providing a composition comprising a glycerolphosphate ester linkage; and, (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide cleaves the glycerolphosphate ester linkage. In one aspect, the conditions comprise between about pH 5 to about 5.5, or, between about pH 4.5 to about 5.0. In one aspect, the conditions comprise a temperature of between about 40°C and about 70°C. In one aspect, the composition comprises a vegetable oil. In one aspect, the composition comprises an oilseed phospholipid. In one aspect, the cleavage reaction can generate a water extractable phosphorylated base and a diglyceride.

The invention provides methods for oil degumming comprising the following steps: (a) providing a polypeptide having a phospholipase activity, wherein the polypeptide comprises an amino acid sequence of the invention, or the polypeptide is encoded by a nucleic acid of the invention; (b) providing a composition comprising a vegetable oil; and,

(c) contacting the polypeptide of step (a) and the vegetable oil of step (b) under conditions wherein the polypeptide can cleave ester linkages in the vegetable oil, thereby degumming the oil. In one aspect, the vegetable oil comprises oilseed. The vegetable oil can comprise palm oil, rapeseed oil, corn oil, soybean oil, canola oil, sesame oil, peanut oil or sunflower oil. In one aspect, the method further comprises addition of a phospholipase of the invention, another phospholipase or a combination thereof.

The invention provides methods for converting a non-hydratable phospholipid to a hydratable form comprising the following steps: (a) providing a polypeptide having a phospholipase activity, wherein the polypeptide comprises an amino acid sequence of the invention, or the polypeptide is encoded by a nucleic acid of the invention; (b) providing a composition comprising a non-hydratable phospholipid; and, (c) contacting the polypeptide of step (a) and the non-hydratable phospholipid of step (b) under conditions wherein the polypeptide can cleave ester linkages in the non-hydratable phospholipid, thereby converting a non-hydratable phospholipid to a hydratable form.

The invention provides methods for degumming an oil comprising the following steps: (a) providing a composition comprising a polypeptide of the invention having a phospholipase activity or a polypeptide encoded by a nucleic acid of the invention; (b) providing an composition comprising a fat or an oil comprising a phospholipid; and (c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the polypeptide can degum the phospholipid-comprising composition (under conditions wherein the polypeptide of the invention can catalyze the hydrolysis of a phospholipid). In one aspect the oil-comprising composition comprises a plant, an animal, an algae or a fish oil. The plant oil can comprise a soybean oil, a rapeseed oil, a corn oil, an oil from a palm kernel, a canola oil, a sunflower oil, a sesame oil or a peanut oil. The polypeptide can hydrolyze a phosphatide from a hydratable and/or a non-hydratable phospholipid in the oil-comprising composition. The polypeptide can hydrolyze a phosphatide at a glyceryl phosphoester bond to generate a diglyceride and water-soluble phosphate compound. The polypeptide can have a phospholipase C, B, A or D activity. In one aspect, a phospholipase D activity and a phosphatase enzyme are added. The contacting can comprise hydrolysis of a hydrated phospholipid in an oil. The hydrolysis conditions of can comprise a temperature of about 20°C to 40°C at an alkaline pH. The alkaline conditions can comprise a pH of about pH 8 to pH 10. The hydrolysis conditions can comprise a reaction time of about 3 to 10 minutes. The hydrolysis conditions can comprise hydrolysis of hydratable and non-hydratable phospholipids in oil at a temperature of about 50°C to 60°C, at

a pH of about pH 5 to pH 6.5 using a reaction time of about 30 to 60 minutes. The polypeptide can be bound to a filter and the phospholipid-containing fat or oil is passed through the filter. The polypeptide can be added to a solution comprising the phospholipid-containing fat or oil and then the solution is passed through a filter.

The invention provides methods for converting a non-hydratable phospholipid to a hydratable form comprising the following steps: (a) providing a composition comprising a polypeptide having a phospholipase activity of the invention, or a polypeptide encoded by a nucleic acid of the invention; (b) providing an composition comprising a non-hydratable phospholipid; and (c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the polypeptide converts the non-hydratable phospholipid to a hydratable form. The polypeptide can have a phospholipase C activity. The polypeptide can have a phospholipase D activity and a phosphatase enzyme is also added.

The invention provides methods for caustic refining of a phospholipid-containing composition comprising the following steps: (a) providing a composition comprising a polypeptide of the invention having a phospholipase activity, or a polypeptide encoded by a nucleic acid of the invention; (b) providing an composition comprising a phospholipid; and (c) contacting the polypeptide of step (a) with the composition of step (b) before, during or after the caustic refining. The polypeptide can have a phospholipase C activity. The polypeptide can be added before caustic refining and the composition comprising the phospholipid can comprise a plant and the polypeptide can be expressed transgenically in the plant, the polypeptide having a phospholipase activity can be added during crushing of a seed or other plant part, or, the polypeptide having a phospholipase activity is added following crushing or prior to refining. The polypeptide can be added during caustic refining and varying levels of acid and caustic can be added depending on levels of phosphorous and levels of free fatty acids. The polypeptide can be added after caustic refining: in an intense mixer or retention mixer prior to separation; following a heating step; in a centrifuge; in a soapstock; in a washwater; or, during bleaching or deodorizing steps.

The invention provides methods for purification of a phytosterol or a triterpene comprising the following steps: (a) providing a composition comprising a polypeptide of the invention having a phospholipase activity, or a polypeptide encoded by a nucleic acid of the invention; (b) providing an composition comprising a phytosterol or a triterpene; and (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the

composition. The polypeptide can have a phospholipase C activity. The phytosterol or a triterpene can comprise a plant sterol. The plant sterol can be derived from a vegetable oil. The vegetable oil can comprise a coconut oil, canola oil, cocoa butter oil, corn oil, cottonseed oil, linseed oil, olive oil, palm oil, peanut oil, oil derived from a rice bran, safflower oil, sesame oil, soybean oil or a sunflower oil. The method can comprise use of nonpolar solvents to quantitatively extract free phytosterols and phytosteryl fatty-acid esters. The phytosterol or a triterpene can comprise a β-sitosterol, a campesterol, a stigmasterol, a stigmastanol, a β-sitostanol, a sitostanol, a desmosterol, a chalinasterol, a poriferasterol, a clionasterol or a brassicasterol.

The invention provides methods for refining a crude oil comprising the following steps: (a) providing a composition comprising a polypeptide of the invention having a phospholipase activity, or a polypeptide encoded by a nucleic acid of the invention; (b) providing a composition comprising an oil comprising a phospholipid; and (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition. The polypeptide can have a phospholipase C activity. The polypeptide can have a phospholipase activity is in a water solution that is added to the composition. The water level can be between about 0.5 to 5%. The process time can be less than about 2 hours, less than about 60 minutes, less than about 30 minutes, less than 15 minutes, or less than 5 minutes. The hydrolysis conditions can comprise a temperature of between about 25°C-70°C. The hydrolysis conditions can comprise use of caustics. The hydrolysis conditions can comprise a pH of between about pH 3 and pH 10, between about pH 4 and pH 9, or between about pH 5 and pH 8. The hydrolysis conditions can comprise addition of emulsifiers and/or mixing after the contacting of step (c). The methods can comprise addition of an emulsion-breaker and/or heat to promote separation of an aqueous phase. The methods can comprise degumming before the contacting step to collect lecithin by centrifugation and then adding a PLC, a PLC and/or a PLA to remove non-hydratable phospholipids. The methods can comprise water degumming of crude oil to less than 10 ppm for edible oils and subsequent physical refining to less than about 50 ppm for biodiesel oils. The methods can comprise addition of acid to promote hydration of non-hydratable phospholipids.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

All publications, patents, patent applications, GenBank sequences and ATCC deposits, cited herein are hereby expressly incorporated by reference for all purposes.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figure 1 is a block diagram of a computer system, as described in detail, below.

Figure 2 is a flow diagram illustrating one aspect of a process 200 for comparing a new nucleotide or protein sequence with a database of sequences in order to determine the homology levels between the new sequence and the sequences in the database, as described in detail, below.

Figure 3 is a flow diagram illustrating one embodiment of a process in a computer for determining whether two sequences are homologous, as described in detail, below.

Figure 4 is a flow diagram illustrating one aspect of an identifier process for detecting the presence of a feature in a sequence, as described in detail, below.

Figures 5A, 5B and 5C schematically illustrate a model two-phase system for simulation of PLC-mediated degumming, as described in detail in Example 2, below.

Figure 6 schematically illustrates an exemplary vegetable oil refining process using the phospholipases of the invention.

Figure 7 schematically illustrates an exemplary degumming process of the invention for physically refined oils, as discussed in detail, below.

Figure 8 schematically illustrates phosphatide hydrolysis with a phospholipase C of the invention, as discussed in detail, below.

Figure 9 schematically illustrates application of a phospholipase C of the invention as a "Caustic Refining Aid" (Long Mix Caustic Refining), as discussed in detail, below.

Figure 10 schematically illustrates application of a phospholipase C of the invention as a degumming aid, as discussed in detail, below.

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides phospholipases (e.g., phospholipase A, B, C, D, patatin enzymes), polynucleotides encoding them and methods for making and using them. The invention provides enzymes that efficiently cleave glycerolphosphate ester linkage in oils, such as vegetable oils, e.g., oilseed phospholipids, to generate a water extractable phosphorylated base and a diglyceride. In one aspect, the phospholipases of the invention have a lipid acyl hydrolase (LAH) activity. In alternative aspects, the phospholipases of the invention can cleave glycerolphosphate ester linkages in phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and sphingomyelin.

A phospholipase of the invention (e.g., phospholipase A, B, C, D, patatin enzymes) can be used for enzymatic degumming of vegetable oils because the phosphate moiety is soluble in water and easy to remove. The diglyceride product will remain in the oil and therefore will reduce losses. The PLCs of the invention can be used in addition to or in place of PLA1s and PLA2s in commercial oil degumming, such as in the ENZYMAX® process, where phospholipids are hydrolyzed by PLA1 and PLA2.

In one aspect, the phospholipases of the invention are active at a high and/or at a low temperature, or, over a wide range of temperature, e.g., they can be active in the temperatures ranging between 20°C to 90°C, between 30°C to 80°C, or between 40°C to 70°C. The invention also provides phospholipases of the invention have activity at alkaline pHs or at acidic pHs, e.g., low water acidity. In alternative aspects, the phospholipases of the invention can have activity in acidic pHs as low as pH 6.5, pH 6.0, pH 5.5, pH 5.0, pH 4.5, pH 4.0 and pH 3.5. In alternative aspects, the phospholipases of the invention can have activity in alkaline pHs as high as pH 7.5, pH 8.0, pH 8.5, pH 9.0, and pH 9.5. In one aspect, the phospholipases of the invention are active in the temperature range of between about 40°C to about 70°C under conditions of low water activity (low water content).

The invention also provides methods for further modifying the exemplary phospholipases of the invention to generate enzymes with desirable properties. For example, phospholipases generated by the methods of the invention can have altered substrate specificities, substrate binding specificities, substrate cleavage patterns, thermal stability, pH/activity profile, pH/stability profile (such as increased stability at low, e.g. pH<6 or pH<5, or high, e.g. pH>9, pH values), stability towards oxidation, Ca^{2+} dependency, specific activity and the like. The invention provides for altering any property of interest. For instance, the alteration may result in a variant which, as compared to a parent phospholipase, has altered pH and temperature activity profile.

In one aspect, the phospholipases of the invention are used in various vegetable oil processing steps, such as in vegetable oil extraction, particularly, in the removal of "phospholipid gums" in a process called "oil degumming," as described herein. The production of vegetable oils from various sources, such as soybeans, rapeseed, peanut, sesame, sunflower and corn. The phospholipase enzymes of the invention can be used in place of PLA, e.g., phospholipase A2, in any vegetable oil processing step.

Definitions

The term "phospholipase" encompasses enzymes having any phospholipase activity, for example, cleaving a glycerolphosphate ester linkage (catalyzing hydrolysis of a glycerolphosphate ester linkage), e.g., in an oil, such as a vegetable oil. The phospholipase activity of the invention can generate a water extractable phosphorylated base and a diglyceride. The phospholipase activity of the invention also includes hydrolysis of glycerolphosphate ester linkages at high temperatures, low temperatures, alkaline pHs and at acidic pHs. The term "a phospholipase activity" also includes cleaving a glycerolphosphate ester to generate a water extractable phosphorylated base and a diglyceride. The term "a phospholipase activity" also includes cutting ester bonds of glycerin and phosphoric acid in phospholipids. The term "a phospholipase activity" also includes other activities, such as the ability to bind to a substrate, such as an oil, e.g. a vegetable oil, substrate also including plant and animal phosphatidylcholines, phosphatidyl-ethanolamines, phosphatidylserines and sphingomyelins. The phospholipase activity can comprise a phospholipase C (PLC) activity, a phospholipase A (PLA) activity, such as a phospholipase A1 or phospholipase A2 activity, a phospholipase B (PLB) activity, such as a phospholipase B1 or phospholipase B2 activity, a phospholipase D (PLD) activity, such as a phospholipase D1 or a phospholipase D2 activity. The phospholipase activity can comprise hydrolysis of a glycoprotein, e.g., as a glycoprotein found in a potato tuber or any plant of the genus *Solanum*, e.g., *Solanum tuberosum*. The phospholipase activity can comprise a patatin enzymatic activity, such as a patatin esterase activity (see, e.g., Jimenez (2002) Biotechnol. Prog. 18:635-640). The phospholipase activity can comprise a lipid acyl hydrolase (LAH) activity.

The term "antibody" includes a peptide or polypeptide derived from, modeled after or substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, capable of specifically binding an antigen or epitope, see, e.g. Fundamental Immunology, Third Edition, W.E. Paul, ed., Raven Press, N.Y. (1993); Wilson (1994) J. Immunol. Methods 175:267-273; Yarmush (1992) J. Biochem. Biophys. Methods

25:85-97. The term antibody includes antigen-binding portions, i.e., "antigen binding sites," (e.g., fragments, subsequences, complementarity determining regions (CDRs)) that retain capacity to bind antigen, including (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Single chain antibodies are also included by reference in the term "antibody."

The terms "array" or "microarray" or "biochip" or "chip" as used herein is a plurality of target elements, each target element comprising a defined amount of one or more polypeptides (including antibodies) or nucleic acids immobilized onto a defined area of a substrate surface, as discussed in further detail, below.

As used herein, the terms "computer," "computer program" and "processor" are used in their broadest general contexts and incorporate all such devices, as described in detail, below.

A "coding sequence of" or a "sequence encodes" a particular polypeptide or protein, is a nucleic acid sequence which is transcribed and translated into a polypeptide or protein when placed under the control of appropriate regulatory sequences.

The term "expression cassette" as used herein refers to a nucleotide sequence which is capable of affecting expression of a structural gene (i.e., a protein coding sequence, such as a phospholipase of the invention) in a host compatible with such sequences. Expression cassettes include at least a promoter operably linked with the polypeptide coding sequence; and, optionally, with other sequences, e.g., transcription termination signals. Additional factors necessary or helpful in effecting expression may also be used, e.g., enhancers. "Operably linked" as used herein refers to linkage of a promoter upstream from a DNA sequence such that the promoter mediates transcription of the DNA sequence. Thus, expression cassettes also include plasmids, expression vectors, recombinant viruses, any form of recombinant "naked DNA" vector, and the like. A "vector" comprises a nucleic acid which can infect, transfect, transiently or permanently transduce a cell. It will be recognized that a vector can be a naked nucleic acid, or a nucleic acid complexed with protein or lipid. The vector optionally comprises viral or bacterial nucleic acids and/or proteins, and/or membranes (e.g., a cell membrane, a viral lipid envelope, etc.). Vectors include, but are not

limited to replicons (e.g., RNA replicons, bacteriophages) to which fragments of DNA may be attached and become replicated. Vectors thus include, but are not limited to RNA, autonomous self-replicating circular or linear DNA or RNA (e.g., plasmids, viruses, and the like, see, e.g., U.S. Patent No. 5,217,879), and includes both the expression and non-expression plasmids. Where a recombinant microorganism or cell culture is described as hosting an "expression vector" this includes both extra-chromosomal circular and linear DNA and DNA that has been incorporated into the host chromosome(s). Where a vector is being maintained by a host cell, the vector may either be stably replicated by the cells during mitosis as an autonomous structure, or is incorporated within the host's genome.

"Plasmids" are designated by a lower case "p" preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described herein are known in the art and will be apparent to the ordinarily skilled artisan.

The term "gene" means the segment of DNA involved in producing a polypeptide chain, including, *inter alia*, regions preceding and following the coding region, such as leader and trailer, promoters and enhancers, as well as, where applicable, intervening sequences (introns) between individual coding segments (exons).

The phrases "nucleic acid" or "nucleic acid sequence" as used herein refer to an oligonucleotide, nucleotide, polynucleotide, or to a fragment of any of these, to DNA or RNA (e.g., mRNA, rRNA, tRNA, iRNA) of genomic or synthetic origin which may be single-stranded or double-stranded and may represent a sense or antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material, natural or synthetic in origin, including, e.g., iRNA, ribonucleoproteins (e.g., double stranded iRNAs, e.g., iRNPs). The term encompasses nucleic acids, i.e., oligonucleotides, containing known analogues of natural nucleotides. The term also encompasses nucleic-acid-like structures with synthetic backbones, see e.g., Mata (1997) Toxicol. Appl. Pharmacol. 144:189-197; Strauss-Soukup (1997) Biochemistry 36:8692-8698; Samstag (1996) Antisense Nucleic Acid Drug Dev 6:153-156.

"Amino acid" or "amino acid sequence" as used herein refer to an oligopeptide, peptide, polypeptide, or protein sequence, or to a fragment, portion, or subunit of any of these, and to naturally occurring or synthetic molecules.

The terms "polypeptide" and "protein" as used herein, refer to amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and

may contain modified amino acids other than the 20 gene-encoded amino acids. The term "polypeptide" also includes peptides and polypeptide fragments, motifs and the like. The term also includes glycosylated polypeptides. The peptides and polypeptides of the invention also include all "mimetic" and "peptidomimetic" forms, as described in further detail, below.

As used herein, the term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment. As used herein, an isolated material or composition can also be a "purified" composition, i.e., it does not require absolute purity; rather, it is intended as a relative definition. Individual nucleic acids obtained from a library can be conventionally purified to electrophoretic homogeneity. In alternative aspects, the invention provides nucleic acids which have been purified from genomic DNA or from other sequences in a library or other environment by at least one, two, three, four, five or more orders of magnitude.

As used herein, the term "recombinant" means that the nucleic acid is adjacent to a "backbone" nucleic acid to which it is not adjacent in its natural environment. In one aspect, nucleic acids represent 5% or more of the number of nucleic acid inserts in a population of nucleic acid "backbone molecules." "Backbone molecules" according to the invention include nucleic acids such as expression vectors, self-replicating nucleic acids, viruses, integrating nucleic acids, and other vectors or nucleic acids used to maintain or manipulate a nucleic acid insert of interest. In one aspect, the enriched nucleic acids represent 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of the number of nucleic acid inserts in the population of recombinant backbone molecules. "Recombinant" polypeptides or proteins refer to polypeptides or proteins produced by recombinant DNA techniques; e.g., produced from cells transformed by an exogenous DNA construct encoding the desired polypeptide or protein. "Synthetic" polypeptides or protein are those prepared by chemical synthesis, as described in further detail, below.

A promoter sequence is "operably linked to" a coding sequence when RNA polymerase which initiates transcription at the promoter will transcribe the coding sequence into mRNA, as discussed further, below.

"Oligonucleotide" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

The phrase "substantially identical" in the context of two nucleic acids or polypeptides, refers to two or more sequences that have at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% nucleotide or amino acid residue (sequence) identity, when compared and aligned for maximum correspondence, as measured using one any known sequence comparison algorithm, as discussed in detail below, or by visual inspection. In alternative aspects, the invention provides nucleic acid and polypeptide sequences having substantial identity to an exemplary sequence of the invention, e.g., SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, etc., over a region of at least about 100 residues, 150 residues, 200 residues, 300 residues, 400 residues, or a region ranging from between about 50 residues to the full length of the nucleic acid or polypeptide. Nucleic acid sequences of the invention can be substantially identical over the entire length of a polypeptide coding region.

Additionally a "substantially identical" amino acid sequence is a sequence that differs from a reference sequence by one or more conservative or non-conservative amino acid substitutions, deletions, or insertions, particularly when such a substitution occurs at a site that is not the active site of the molecule, and provided that the polypeptide essentially retains its functional properties. A conservative amino acid substitution, for example, substitutes one amino acid for another of the same class (e.g., substitution of one hydrophobic amino acid, such as isoleucine, valine, leucine, or methionine, for another, or substitution of one polar amino acid for another, such as substitution of arginine for lysine, glutamic acid for aspartic acid or glutamine for asparagine). One or more amino acids can be deleted, for example, from a phospholipase polypeptide, resulting in modification of the structure of the polypeptide, without significantly altering its biological activity. For example, amino- or carboxyl-terminal amino acids that are not required for phospholipase biological activity can be removed. Modified polypeptide sequences of the invention can be assayed for phospholipase biological activity by any number of methods, including contacting the modified polypeptide sequence with a phospholipase substrate and determining whether the modified polypeptide decreases the amount of specific substrate in the assay or increases the

bioproducts of the enzymatic reaction of a functional phospholipase with the substrate, as discussed further, below.

"Hybridization" refers to the process by which a nucleic acid strand joins with a complementary strand through base pairing. Hybridization reactions can be sensitive and selective so that a particular sequence of interest can be identified even in samples in which it is present at low concentrations. Suitably stringent conditions can be defined by, for example, the concentrations of salt or formamide in the prehybridization and hybridization solutions, or by the hybridization temperature, and are well known in the art. For example, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature, altering the time of hybridization, as described in detail, below. In alternative aspects, nucleic acids of the invention are defined by their ability to hybridize under various stringency conditions (e.g., high, medium, and low), as set forth herein.

The term "variant" refers to polynucleotides or polypeptides of the invention modified at one or more base pairs, codons, introns, exons, or amino acid residues (respectively) yet still retain the biological activity of a phospholipase of the invention. Variants can be produced by any number of means included methods such as, for example, error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, GSSM and any combination thereof. Techniques for producing variant phospholipases having activity at a pH or temperature, for example, that is different from a wild-type phospholipase, are included herein.

The term "saturation mutagenesis" or "GSSM" includes a method that uses degenerate oligonucleotide primers to introduce point mutations into a polynucleotide, as described in detail, below.

The term "optimized directed evolution system" or "optimized directed evolution" includes a method for reassembling fragments of related nucleic acid sequences, e.g., related genes, and explained in detail, below.

The term "synthetic ligation reassembly" or "SLR" includes a method of ligating oligonucleotide fragments in a non-stochastic fashion, and explained in detail, below.

<u>Generating and Manipulating Nucleic Acids</u>

The invention provides nucleic acids (e.g., the exemplary SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105), including expression cassettes such as expression vectors, encoding the polypeptides and phospholipases of the invention. The invention also includes methods for discovering new phospholipase sequences using the nucleic acids of the invention. Also provided are methods for modifying the nucleic acids of the invention by, e.g., synthetic ligation reassembly, optimized directed evolution system and/or saturation mutagenesis.

The nucleic acids of the invention can be made, isolated and/or manipulated by, e.g., cloning and expression of cDNA libraries, amplification of message or genomic DNA by PCR, and the like. In practicing the methods of the invention, homologous genes can be modified by manipulating a template nucleic acid, as described herein. The invention can be practiced in conjunction with any method or protocol or device known in the art, which are well described in the scientific and patent literature.

General Techniques

The nucleic acids used to practice this invention, whether RNA, iRNA, antisense nucleic acid, cDNA, genomic DNA, vectors, viruses or hybrids thereof, may be isolated from a variety of sources, genetically engineered, amplified, and/or expressed/ generated recombinantly. Recombinant polypeptides generated from these nucleic acids can be individually isolated or cloned and tested for a desired activity. Any recombinant expression system can be used, including bacterial, mammalian, yeast, insect or plant cell expression systems.

Alternatively, these nucleic acids can be synthesized *in vitro* by well-known chemical synthesis techniques, as described in, e.g., Adams (1983) J. Am. Chem. Soc. 105:661; Belousov (1997) Nucleic Acids Res. 25:3440-3444; Frenkel (1995) Free Radic.

Biol. Med. 19:373-380; Blommers (1994) Biochemistry 33:7886-7896; Narang (1979) Meth. Enzymol. 68:90; Brown (1979) Meth. Enzymol. 68:109; Beaucage (1981) Tetra. Lett. 22:1859; U.S. Patent No. 4,458,066.

Techniques for the manipulation of nucleic acids, such as, e.g., subcloning, labeling probes (e.g., random-primer labeling using Klenow polymerase, nick translation, amplification), sequencing, hybridization and the like are well described in the scientific and patent literature, see, e.g., Sambrook, ed., MOLECULAR CLONING: A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel, ed. John Wiley & Sons, Inc., New York (1997); LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY: HYBRIDIZATION WITH NUCLEIC ACID PROBES, Part I. Theory and Nucleic Acid Preparation, Tijssen, ed. Elsevier, N.Y. (1993).

Another useful means of obtaining and manipulating nucleic acids used to practice the methods of the invention is to clone from genomic samples, and, if desired, screen and re-clone inserts isolated or amplified from, e.g., genomic clones or cDNA clones. Sources of nucleic acid used in the methods of the invention include genomic or cDNA libraries contained in, e.g., mammalian artificial chromosomes (MACs), see, e.g., U.S. Patent Nos. 5,721,118; 6,025,155; human artificial chromosomes, see, e.g., Rosenfeld (1997) Nat. Genet. 15:333-335; yeast artificial chromosomes (YAC); bacterial artificial chromosomes (BAC); P1 artificial chromosomes, see, e.g., Woon (1998) Genomics 50:306-316; P1-derived vectors (PACs), see, e.g., Kern (1997) Biotechniques 23:120-124; cosmids, recombinant viruses, phages or plasmids.

In one aspect, a nucleic acid encoding a polypeptide of the invention is assembled in appropriate phase with a leader sequence capable of directing secretion of the translated polypeptide or fragment thereof.

The invention provides fusion proteins and nucleic acids encoding them. A polypeptide of the invention can be fused to a heterologous peptide or polypeptide, such as N-terminal identification peptides which impart desired characteristics, such as increased stability or simplified purification. Peptides and polypeptides of the invention can also be synthesized and expressed as fusion proteins with one or more additional domains linked thereto for, e.g., producing a more immunogenic peptide, to more readily isolate a recombinantly synthesized peptide, to identify and isolate antibodies and antibody-expressing B cells, and the like. Detection and purification facilitating domains include, e.g., metal chelating peptides such as polyhistidine tracts and histidine-tryptophan modules that allow

purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle WA). The inclusion of a cleavable linker sequences such as Factor Xa or enterokinase (Invitrogen, San Diego CA) between a purification domain and the motif-comprising peptide or polypeptide to facilitate purification. For example, an expression vector can include an epitope-encoding nucleic acid sequence linked to six histidine residues followed by a thioredoxin and an enterokinase cleavage site (see e.g., Williams (1995) Biochemistry 34:1787-1797; Dobeli (1998) Protein Expr. Purif. 12:404-414). The histidine residues facilitate detection and purification while the enterokinase cleavage site provides a means for purifying the epitope from the remainder of the fusion protein. Technology pertaining to vectors encoding fusion proteins and application of fusion proteins are well described in the scientific and patent literature, see e.g., Kroll (1993) DNA Cell. Biol., 12:441-53.

Transcriptional and translational control sequences

The invention provides nucleic acid (e.g., DNA) sequences of the invention operatively linked to expression (e.g., transcriptional or translational) control sequence(s),. e.g., promoters or enhancers, to direct or modulate RNA synthesis/ expression. The expression control sequence can be in an expression vector. Exemplary bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda PR, PL and trp. Exemplary eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein I.

Promoters suitable for expressing a polypeptide in bacteria include the *E. coli* lac or trp promoters, the lacI promoter, the lacZ promoter, the T3 promoter, the T7 promoter, the gpt promoter, the lambda PR promoter, the lambda PL promoter, promoters from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), and the acid phosphatase promoter. Eukaryotic promoters include the CMV immediate early promoter, the HSV thymidine kinase promoter, heat shock promoters, the early and late SV40 promoter, LTRs from retroviruses, and the mouse metallothionein-I promoter. Other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses may also be used.

Expression vectors and cloning vehicles

The invention provides expression vectors and cloning vehicles comprising nucleic acids of the invention, e.g., sequences encoding the phospholipases of the invention.

Expression vectors and cloning vehicles of the invention can comprise viral particles, baculovirus, phage, plasmids, phagemids, cosmids, fosmids, bacterial artificial chromosomes, viral DNA (e.g., vaccinia, adenovirus, foul pox virus, pseudorabies and derivatives of SV40), P1-based artificial chromosomes, yeast plasmids, yeast artificial chromosomes, and any other vectors specific for specific hosts of interest (such as bacillus, Aspergillus and yeast). Vectors of the invention can include chromosomal, non-chromosomal and synthetic DNA sequences. Large numbers of suitable vectors are known to those of skill in the art, and are commercially available. Exemplary vectors are include: bacterial: pQE vectors (Qiagen), pBluescript plasmids, pNH vectors, (lambda-ZAP vectors (Stratagene); ptrc99a, pKK223-3, pDR540, pRIT2T (Pharmacia); Eukaryotic: pXT1, pSG5 (Stratagene), pSVK3, pBPV, pMSG, pSVLSV40 (Pharmacia). However, any other plasmid or other vector may be used so long as they are replicable and viable in the host. Low copy number or high copy number vectors may be employed with the present invention.

The expression vector may comprise a promoter, a ribosome-binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression. Mammalian expression vectors can comprise an origin of replication, any necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences. In some aspects, DNA sequences derived from the SV40 splice and polyadenylation sites may be used to provide the required non-transcribed genetic elements.

In one aspect, the expression vectors contain one or more selectable marker genes to permit selection of host cells containing the vector. Such selectable markers include genes encoding dihydrofolate reductase or genes conferring neomycin resistance for eukaryotic cell culture, genes conferring tetracycline or ampicillin resistance in *E. coli*, and the *S. cerevisiae* TRP1 gene. Promoter regions can be selected from any desired gene using chloramphenicol transferase (CAT) vectors or other vectors with selectable markers.

Vectors for expressing the polypeptide or fragment thereof in eukaryotic cells may also contain enhancers to increase expression levels. Enhancers are *cis*-acting elements of DNA, usually from about 10 to about 300 bp in length that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin bp 100 to 270, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and the adenovirus enhancers.

A DNA sequence may be inserted into a vector by a variety of procedures. In general, the DNA sequence is ligated to the desired position in the vector following digestion

of the insert and the vector with appropriate restriction endonucleases. Alternatively, blunt ends in both the insert and the vector may be ligated. A variety of cloning techniques are known in the art, e.g., as described in Ausubel and Sambrook. Such procedures and others are deemed to be within the scope of those skilled in the art.

The vector may be in the form of a plasmid, a viral particle, or a phage. Other vectors include chromosomal, non-chromosomal and synthetic DNA sequences, derivatives of SV40; bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. A variety of cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by, e.g., Sambrook.

Particular bacterial vectors which may be used include the commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017), pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden), GEM1 (Promega Biotec, Madison, WI, USA) pQE70, pQE60, pQE-9 (Qiagen), pD10, psiX174 pBluescript II KS, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene), ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia), pKK232-8 and pCM7. Particular eukaryotic vectors include pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, and pSVL (Pharmacia). However, any other vector may be used as long as it is replicable and viable in the host cell.

Host cells and transformed cells

The invention also provides a transformed cell comprising a nucleic acid sequence of the invention, e.g., a sequence encoding a phospholipase of the invention, a vector of the invention. The host cell may be any of the host cells familiar to those skilled in the art, including prokaryotic cells, eukaryotic cells, such as bacterial cells, fungal cells, yeast cells, mammalian cells, insect cells, or plant cells. Exemplary bacterial cells include *E. coli*, *Streptomyces*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*. Exemplary insect cells include *Drosophila* S2 and *Spodoptera* Sf9. Exemplary animal cells include CHO, COS or Bowes melanoma or any mouse or human cell line. The selection of an appropriate host is within the abilities of those skilled in the art.

The vector may be introduced into the host cells using any of a variety of techniques, including transformation, transfection, transduction, viral infection, gene guns, or

Ti-mediated gene transfer. Particular methods include calcium phosphate transfection, DEAE-Dextran mediated transfection, lipofection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

Where appropriate, the engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes of the invention. Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter may be induced by appropriate means (e.g., temperature shift or chemical induction) and the cells may be cultured for an additional period to allow them to produce the desired polypeptide or fragment thereof.

Cells can be harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract is retained for further purification. Microbial cells employed for expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well known to those skilled in the art. The expressed polypeptide or fragment thereof can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the polypeptide. If desired, high performance liquid chromatography (HPLC) can be employed for final purification steps.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts and other cell lines capable of expressing proteins from a compatible vector, such as the C127, 3T3, CHO, HeLa and BHK cell lines.

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Depending upon the host employed in a recombinant production procedure, the polypeptides produced by host cells containing the vector may be glycosylated or may be non-glycosylated. Polypeptides of the invention may or may not also include an initial methionine amino acid residue.

Cell-free translation systems can also be employed to produce a polypeptide of the invention. Cell-free translation systems can use mRNAs transcribed from a DNA construct comprising a promoter operably linked to a nucleic acid encoding the polypeptide

or fragment thereof. In some aspects, the DNA construct may be linearized prior to conducting an *in vitro* transcription reaction. The transcribed mRNA is then incubated with an appropriate cell-free translation extract, such as a rabbit reticulocyte extract, to produce the desired polypeptide or fragment thereof.

The expression vectors can contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli*.

Amplification of Nucleic Acids

In practicing the invention, nucleic acids encoding the polypeptides of the invention, or modified nucleic acids, can be reproduced by, e.g., amplification. The invention provides amplification primer sequence pairs for amplifying nucleic acids encoding polypeptides with a phospholipase activity. In one aspect, the primer pairs are capable of amplifying nucleic acid sequences of the invention, e.g., including the exemplary SEQ ID NO:1, or a subsequence thereof; a sequence as set forth in SEQ ID NO:3, or a subsequence thereof; a sequence as set forth in SEQ ID NO:5, or a subsequence thereof; and, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, etc. One of skill in the art can design amplification primer sequence pairs for any part of or the full length of these sequences.

The invention provides an amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having a phospholipase activity, wherein the primer pair is capable of amplifying a nucleic acid comprising a sequence of the invention, or fragments or subsequences thereof. One or each member of the amplification primer sequence pair can comprise an oligonucleotide comprising at least about 10 to 50 consecutive bases of the sequence, or about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 consecutive bases of the sequence.

The invention provides amplification primer pairs, wherein the primer pair comprises a first member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues of a nucleic acid of the invention, and a second member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues of the complementary strand of the first member. The invention provides phospholipases generated by amplification, e.g., polymerase chain reaction (PCR), using an amplification primer pair of the invention. The invention provides methods of making a phospholipase by amplification, e.g., polymerase chain reaction (PCR),

using an amplification primer pair of the invention. In one aspect, the amplification primer pair amplifies a nucleic acid from a library, e.g., a gene library, such as an environmental library.

Amplification reactions can also be used to quantify the amount of nucleic acid in a sample (such as the amount of message in a cell sample), label the nucleic acid (e.g., to apply it to an array or a blot), detect the nucleic acid, or quantify the amount of a specific nucleic acid in a sample. In one aspect of the invention, message isolated from a cell or a cDNA library are amplified. The skilled artisan can select and design suitable oligonucleotide amplification primers. Amplification methods are also well known in the art, and include, *e.g.*, polymerase chain reaction, PCR (see, e.g., PCR PROTOCOLS, A GUIDE TO METHODS AND APPLICATIONS, ed. Innis, Academic Press, N.Y. (1990) and PCR STRATEGIES (1995), ed. Innis, Academic Press, Inc., N.Y., ligase chain reaction (LCR) (see, e.g., Wu (1989) Genomics 4:560; Landegren (1988) Science 241:1077; Barringer (1990) Gene 89:117); transcription amplification (see, e.g., Kwoh (1989) Proc. Natl. Acad. Sci. USA 86:1173); and, self-sustained sequence replication (see, e.g., Guatelli (1990) Proc. Natl. Acad. Sci. USA 87:1874); Q Beta replicase amplification (see, e.g., Smith (1997) J. Clin. Microbiol. 35:1477-1491), automated Q-beta replicase amplification assay (see, e.g., Burg (1996) Mol. Cell. Probes 10:257-271) and other RNA polymerase mediated techniques (*e.g.*, NASBA, Cangene, Mississauga, Ontario); see also Berger (1987) Methods Enzymol. 152:307-316; Sambrook; Ausubel; U.S. Patent Nos. 4,683,195 and 4,683,202; Sooknanan (1995) Biotechnology 13:563-564.

Determining the degree of sequence identity

The invention provides nucleic acids comprising sequences having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to an exemplary nucleic acid of the invention (e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63,

SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, and nucleic acids encoding SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106) over a region of at least about 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550 or more, residues. The invention provides polypeptides comprising sequences having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to an exemplary polypeptide of the invention. The extent of sequence identity (homology) may be determined using any computer program and associated parameters, including those described herein, such as BLAST 2.2.2. or FASTA version 3.0t78, with the default parameters.

In alternative embodiments, the sequence identify can be over a region of at least about 5, 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400 consecutive residues, or the full length of the nucleic acid or polypeptide. The extent of sequence identity (homology) may be determined using any computer program and associated parameters, including those described herein, such as BLAST 2.2.2. or FASTA version 3.0t78, with the default parameters.

Homologous sequences also include RNA sequences in which uridines replace the thymines in the nucleic acid sequences. The homologous sequences may be obtained using any of the procedures described herein or may result from the correction of a sequencing error. It will be appreciated that the nucleic acid sequences as set forth herein can

be represented in the traditional single character format (see, e.g., Stryer, Lubert. Biochemistry, 3rd Ed., W. H Freeman & Co., New York) or in any other format which records the identity of the nucleotides in a sequence.

Various sequence comparison programs identified herein are used in this aspect of the invention. Protein and/or nucleic acid sequence identities (homologies) may be evaluated using any of the variety of sequence comparison algorithms and programs known in the art. Such algorithms and programs include, but are not limited to, TBLASTN, BLASTP, FASTA, TFASTA, and CLUSTALW (Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85(8):2444-2448, 1988; Altschul et al., J. Mol. Biol. 215(3):403-410, 1990; Thompson et al., Nucleic Acids Res. 22(2):4673-4680, 1994; Higgins et al., Methods Enzymol. 266:383-402, 1996; Altschul et al., J. Mol. Biol. 215(3):403-410, 1990; Altschul et al., Nature Genetics 3:266-272, 1993).

Homology or identity can be measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software matches similar sequences by assigning degrees of homology to various deletions, substitutions and other modifications. The terms "homology" and "identity" in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same when compared and aligned for maximum correspondence over a comparison window or designated region as measured using any number of sequence comparison algorithms or by manual alignment and visual inspection. For sequence comparison, one sequence can act as a reference sequence (an exemplary sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, etc.) to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous residues. For example, in alternative aspects of the invention, continugous residues ranging anywhere from 20 to the full length of an exemplary

sequence of the invention, e.g., SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, etc., are compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. If the reference sequence has the requisite sequence identity to an exemplary sequence of the invention, e.g., 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to a sequence of the invention, e.g., SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, etc., that sequence is within the scope of the invention. In alternative embodiments, subsequences ranging from about 20 to 600, about 50 to 200, and about 100 to 150 are compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequence for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482, 1981, by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443, 1970, by the search for similarity method of person & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection. Other algorithms for determining homology or identity include, for example, in addition to a BLAST program (Basic Local Alignment Search Tool at the National Center for Biological Information), ALIGN, AMAS (Analysis of Multiply Aligned Sequences), AMPS (Protein Multiple Sequence Alignment), ASSET (Aligned Segment Statistical Evaluation Tool), BANDS, BESTSCOR, BIOSCAN (Biological Sequence Comparative Analysis Node), BLIMPS (BLocks IMProved Searcher), FASTA, Intervals & Points, BMB, CLUSTAL V, CLUSTAL W, CONSENSUS, LCONSENSUS, WCONSENSUS, Smith-Waterman algorithm, DARWIN, Las Vegas algorithm, FNAT (Forced Nucleotide Alignment Tool), Framealign, Framesearch, DYNAMIC, FILTER, FSAP (Fristensky Sequence Analysis Package), GAP (Global Alignment Program), GENAL, GIBBS, GenQuest, ISSC (Sensitive Sequence Comparison), LALIGN (Local Sequence Alignment), LCP (Local Content Program), MACAW (Multiple Alignment Construction & Analysis Workbench), MAP (Multiple Alignment Program), MBLKP, MBLKN, PIMA (Pattern-Induced Multi-sequence Alignment), SAGA (Sequence Alignment by Genetic

Algorithm) and WHAT-IF. Such alignment programs can also be used to screen genome databases to identify polynucleotide sequences having substantially identical sequences. A number of genome databases are available, for example, a substantial portion of the human genome is available as part of the Human Genome Sequencing Project (Gibbs, 1995). Several genomes have been sequenced, e.g., *M. genitalium* (Fraser et al., 1995), *M. jannaschii* (Bult et al., 1996), *H. influenzae* (Fleischmann et al., 1995), *E. coli* (Blattner et al., 1997), and yeast (S. cerevisiae) (Mewes et al., 1997), and *D. melanogaster* (Adams et al., 2000). Significant progress has also been made in sequencing the genomes of model organism, such as mouse, *C. elegans*, and *Arabadopsis sp*. Databases containing genomic information annotated with some functional information are maintained by different organization, and are accessible via the internet.

BLAST, BLAST 2.0 and BLAST 2.2.2 algorithms are also used to practice the invention. They are described, e.g., in Altschul (1977) Nuc. Acids Res. 25:3389-3402; Altschul (1990) J. Mol. Biol. 215:403-410. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul (1990) supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectations (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N= -4, and a comparison of both strands. The BLAST algorithm

also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873). One measure of similarity provided by BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a references sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001. In one aspect, protein and nucleic acid sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST"). For example, five specific BLAST programs can be used to perform the following task: (1) BLASTP and BLAST3 compare an amino acid query sequence against a protein sequence database; (2) BLASTN compares a nucleotide query sequence against a nucleotide sequence database; (3) BLASTX compares the six-frame conceptual translation products of a query nucleotide sequence (both strands) against a protein sequence database; (4) TBLASTN compares a query protein sequence against a nucleotide sequence database translated in all six reading frames (both strands); and, (5) TBLASTX compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database. The BLAST programs identify homologous sequences by identifying similar segments, which are referred to herein as "high-scoring segment pairs," between a query amino or nucleic acid sequence and a test sequence which is preferably obtained from a protein or nucleic acid sequence database. High-scoring segment pairs are preferably identified (i.e., aligned) by means of a scoring matrix, many of which are known in the art. Preferably, the scoring matrix used is the BLOSUM62 matrix (Gonnet et al., Science 256:1443-1445, 1992; Henikoff and Henikoff, Proteins 17:49-61, 1993). Less preferably, the PAM or PAM250 matrices may also be used (see, e.g., Schwartz and Dayhoff, eds., 1978, Matrices for Detecting Distance Relationships: Atlas of Protein Sequence and Structure, Washington: National Biomedical Research Foundation).

In one aspect of the invention, to determine if a nucleic acid has the requisite sequence identity to be within the scope of the invention, the NCBI BLAST 2.2.2 programs is used. default options to blastp. There are about 38 setting options in the BLAST 2.2.2 program. In this exemplary aspect of the invention, all default values are used except for the default filtering setting (i.e., all parameters set to default except filtering which is set to OFF); in its place a "-F F" setting is used, which disables filtering. Use of default filtering often results in Karlin-Altschul violations due to short length of sequence.

The default values used in this exemplary aspect of the invention include:

"Filter for low complexity: ON

> Word Size: 3

> Matrix: Blosum62

> Gap Costs: Existence:11

> Extension:1"

Other default settings are: filter for low complexity OFF, word size of 3 for protein, BLOSUM62 matrix, gap existence penalty of -11 and a gap extension penalty of -1.

An exemplary NCBI BLAST 2.2.2 program setting is set forth in Example 1, below. Note that the "-W" option defaults to 0. This means that, if not set, the word size defaults to 3 for proteins and 11 for nucleotides.

Computer systems and computer program products

To determine and identify sequence identities, structural homologies, motifs and the like *in silico* the sequence of the invention can be stored, recorded, and manipulated on any medium which can be read and accessed by a computer. Accordingly, the invention provides computers, computer systems, computer readable mediums, computer programs products and the like recorded or stored thereon the nucleic acid and polypeptide sequences of the invention, e.g., an exemplary sequence of the invention, e.g., SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, etc. As used herein, the words "recorded" and "stored" refer to a process for storing information on a computer medium. A skilled artisan can readily adopt any known methods for recording information on a computer readable medium to generate manufactures comprising one or more of the nucleic acid and/or polypeptide sequences of the invention.

Another aspect of the invention is a computer readable medium having recorded thereon at least one nucleic acid and/or polypeptide sequence of the invention. Computer readable media include magnetically readable media, optically readable media, electronically readable media and magnetic/optical media. For example, the computer readable media may be a hard disk, a floppy disk, a magnetic tape, CD-ROM, Digital Versatile Disk (DVD), Random Access Memory (RAM), or Read Only Memory (ROM) as well as other types of other media known to those skilled in the art.

Aspects of the invention include systems (e.g., internet based systems), particularly computer systems, which store and manipulate the sequences and sequence information described herein. One example of a computer system 100 is illustrated in block

diagram form in Figure 1. As used herein, "a computer system" refers to the hardware components, software components, and data storage components used to analyze a nucleotide or polypeptide sequence of the invention. The computer system **100** can include a processor for processing, accessing and manipulating the sequence data. The processor **105** can be any well-known type of central processing unit, such as, for example, the Pentium III from Intel Corporation, or similar processor from Sun, Motorola, Compaq, AMD or International Business Machines. The computer system **100** is a general purpose system that comprises the processor 105 and one or more internal data storage components **110** for storing data, and one or more data retrieving devices for retrieving the data stored on the data storage components. A skilled artisan can readily appreciate that any one of the currently available computer systems are suitable.

In one aspect, the computer system **100** includes a processor **105** connected to a bus which is connected to a main memory **115** (preferably implemented as RAM) and one or more internal data storage devices **110**, such as a hard drive and/or other computer readable media having data recorded thereon. The computer system **100** can further include one or more data retrieving device **118** for reading the data stored on the internal data storage devices **110**.

The data retrieving device **118** may represent, for example, a floppy disk drive, a compact disk drive, a magnetic tape drive, or a modem capable of connection to a remote data storage system (e.g., via the internet) etc. In some embodiments, the internal data storage device **110** is a removable computer readable medium such as a floppy disk, a compact disk, a magnetic tape, etc. containing control logic and/or data recorded thereon. The computer system **100** may advantageously include or be programmed by appropriate software for reading the control logic and/or the data from the data storage component once inserted in the data retrieving device.

The computer system **100** includes a display **120** which is used to display output to a computer user. It should also be noted that the computer system **100** can be linked to other computer systems **125a-c** in a network or wide area network to provide centralized access to the computer system **100**. Software for accessing and processing the nucleotide or amino acid sequences of the invention can reside in main memory 115 during execution.

In some aspects, the computer system **100** may further comprise a sequence comparison algorithm for comparing a nucleic acid sequence of the invention. The algorithm and sequence(s) can be stored on a computer readable medium. A "sequence comparison algorithm" refers to one or more programs which are implemented (locally or remotely) on

the computer system **100** to compare a nucleotide sequence with other nucleotide sequences and/or compounds stored within a data storage means. For example, the sequence comparison algorithm may compare the nucleotide sequences of an exemplary sequence, e.g., SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, etc. stored on a computer readable medium to reference sequences stored on a computer readable medium to identify homologies or structural motifs.

The parameters used with the above algorithms may be adapted depending on the sequence length and degree of homology studied. In some aspects, the parameters may be the default parameters used by the algorithms in the absence of instructions from the user. Figure 2 is a flow diagram illustrating one aspect of a process **200** for comparing a new nucleotide or protein sequence with a database of sequences in order to determine the homology levels between the new sequence and the sequences in the database. The database of sequences can be a private database stored within the computer system **100**, or a public database such as GENBANK that is available through the Internet. The process **200** begins at a start state **201** and then moves to a state **202** wherein the new sequence to be compared is stored to a memory in a computer system **100**. As discussed above, the memory could be any type of memory, including RAM or an internal storage device.

The process **200** then moves to a state **204** wherein a database of sequences is opened for analysis and comparison. The process **200** then moves to a state **206** wherein the first sequence stored in the database is read into a memory on the computer. A comparison is then performed at a state **210** to determine if the first sequence is the same as the second sequence. It is important to note that this step is not limited to performing an exact comparison between the new sequence and the first sequence in the database. Well-known methods are known to those of skill in the art for comparing two nucleotide or protein sequences, even if they are not identical. For example, gaps can be introduced into one sequence in order to raise the homology level between the two tested sequences. The parameters that control whether gaps or other features are introduced into a sequence during comparison are normally entered by the user of the computer system.

Once a comparison of the two sequences has been performed at the state **210**, a determination is made at a decision state **210** whether the two sequences are the same. Of course, the term "same" is not limited to sequences that are absolutely identical. Sequences that are within the homology parameters entered by the user will be marked as "same" in the process **200**. If a determination is made that the two sequences are the same, the process **200** moves to a state **214** wherein the name of the sequence from the database is displayed to the

user. This state notifies the user that the sequence with the displayed name fulfills the homology constraints that were entered. Once the name of the stored sequence is displayed to the user, the process **200** moves to a decision state 218 wherein a determination is made whether more sequences exist in the database. If no more sequences exist in the database, then the process **200** terminates at an end state **220**. However, if more sequences do exist in the database, then the process **200** moves to a state **224** wherein a pointer is moved to the next sequence in the database so that it can be compared to the new sequence. In this manner, the new sequence is aligned and compared with every sequence in the database.

It should be noted that if a determination had been made at the decision state **212** that the sequences were not homologous, then the process **200** would move immediately to the decision state **218** in order to determine if any other sequences were available in the database for comparison. Accordingly, one aspect of the invention is a computer system comprising a processor, a data storage device having stored thereon a nucleic acid sequence of the invention and a sequence comparer for conducting the comparison. The sequence comparer may indicate a homology level between the sequences compared or identify structural motifs, or it may identify structural motifs in sequences which are compared to these nucleic acid codes and polypeptide codes.

Figure 3 is a flow diagram illustrating one embodiment of a process **250** in a computer for determining whether two sequences are homologous. The process **250** begins at a start state **252** and then moves to a state **254** wherein a first sequence to be compared is stored to a memory. The second sequence to be compared is then stored to a memory at a state **256**. The process **250** then moves to a state **260** wherein the first character in the first sequence is read and then to a state **262** wherein the first character of the second sequence is read. It should be understood that if the sequence is a nucleotide sequence, then the character would normally be either A, T, C, G or U. If the sequence is a protein sequence, then it can be a single letter amino acid code so that the first and sequence sequences can be easily compared. A determination is then made at a decision state **264** whether the two characters are the same. If they are the same, then the process **250** moves to a state **268** wherein the next characters in the first and second sequences are read. A determination is then made whether the next characters are the same. If they are, then the process **250** continues this loop until two characters are not the same. If a determination is made that the next two characters are not the same, the process **250** moves to a decision state **274** to determine whether there are any more characters either sequence to read. If there are not any more characters to read, then the process **250** moves to a state **276** wherein the level of homology between the first

and second sequences is displayed to the user. The level of homology is determined by calculating the proportion of characters between the sequences that were the same out of the total number of sequences in the first sequence. Thus, if every character in a first 100 nucleotide sequence aligned with a every character in a second sequence, the homology level would be 100%.

Alternatively, the computer program can compare a reference sequence to a sequence of the invention to determine whether the sequences differ at one or more positions. The program can record the length and identity of inserted, deleted or substituted nucleotides or amino acid residues with respect to the sequence of either the reference or the invention. The computer program may be a program which determines whether a reference sequence contains a single nucleotide polymorphism (SNP) with respect to a sequence of the invention, or, whether a sequence of the invention comprises a SNP of a known sequence. Thus, in some aspects, the computer program is a program which identifies SNPs. The method may be implemented by the computer systems described above and the method illustrated in Figure 3. The method can be performed by reading a sequence of the invention and the reference sequences through the use of the computer program and identifying differences with the computer program.

In other aspects the computer based system comprises an identifier for identifying features within a nucleic acid or polypeptide of the invention. An "identifier" refers to one or more programs which identifies certain features within a nucleic acid sequence. For example, an identifier may comprise a program which identifies an open reading frame (ORF) in a nucleic acid sequence. Figure 4 is a flow diagram illustrating one aspect of an identifier process **300** for detecting the presence of a feature in a sequence. The process **300** begins at a start state **302** and then moves to a state **304** wherein a first sequence that is to be checked for features is stored to a memory **115** in the computer system **100**. The process **300** then moves to a state **306** wherein a database of sequence features is opened. Such a database would include a list of each feature's attributes along with the name of the feature. For example, a feature name could be "Initiation Codon" and the attribute would be "ATG". Another example would be the feature name "TAATAA Box" and the feature attribute would be "TAATAA". An example of such a database is produced by the University of Wisconsin Genetics Computer Group. Alternatively, the features may be structural polypeptide motifs such as alpha helices, beta sheets, or functional polypeptide motifs such as enzymatic active sites, helix-turn-helix motifs or other motifs known to those skilled in the art. Once the database of features is opened at the state **306**, the process **300** moves to a state

308 wherein the first feature is read from the database. A comparison of the attribute of the first feature with the first sequence is then made at a state 310. A determination is then made at a decision state 316 whether the attribute of the feature was found in the first sequence. If the attribute was found, then the process 300 moves to a state 318 wherein the name of the found feature is displayed to the user. The process 300 then moves to a decision state 320 wherein a determination is made whether move features exist in the database. If no more features do exist, then the process 300 terminates at an end state 324. However, if more features do exist in the database, then the process 300 reads the next sequence feature at a state 326 and loops back to the state 310 wherein the attribute of the next feature is compared against the first sequence. If the feature attribute is not found in the first sequence at the decision state 316, the process 300 moves directly to the decision state 320 in order to determine if any more features exist in the database. Thus, in one aspect, the invention provides a computer program that identifies open reading frames (ORFs).

A polypeptide or nucleic acid sequence of the invention may be stored and manipulated in a variety of data processor programs in a variety of formats. For example, a sequence can be stored as text in a word processing file, such as MicrosoftWORD or WORDPERFECT or as an ASCII file in a variety of database programs familiar to those of skill in the art, such as DB2, SYBASE, or ORACLE. In addition, many computer programs and databases may be used as sequence comparison algorithms, identifiers, or sources of reference nucleotide sequences or polypeptide sequences to be compared to a nucleic acid sequence of the invention. The programs and databases used to practice the invention include, but are not limited to: MacPattern (EMBL), DiscoveryBase (Molecular Applications Group), GeneMine (Molecular Applications Group), Look (Molecular Applications Group), MacLook (Molecular Applications Group), BLAST and BLAST2 (NCBI), BLASTN and BLASTX (Altschul et al, J. Mol. Biol. 215: 403, 1990), FASTA (Pearson and Lipman, Proc. Natl. Acad. Sci. USA, 85: 2444, 1988), FASTDB (Brutlag et al. Comp. App. Biosci. 6:237-245, 1990), Catalyst (Molecular Simulations Inc.), Catalyst/SHAPE (Molecular Simulations Inc.), Cerius2.DBAccess (Molecular Simulations Inc.), HypoGen (Molecular Simulations Inc.), Insight II, (Molecular Simulations Inc.), Discover (Molecular Simulations Inc.), CHARMm (Molecular Simulations Inc.), Felix (Molecular Simulations Inc.), DelPhi, (Molecular Simulations Inc.), QuanteMM, (Molecular Simulations Inc.), Homology (Molecular Simulations Inc.), Modeler (Molecular Simulations Inc.), ISIS (Molecular Simulations Inc.), Quanta/Protein Design (Molecular Simulations Inc.), WebLab (Molecular Simulations Inc.), WebLab Diversity Explorer (Molecular Simulations Inc.), Gene Explorer

(Molecular Simulations Inc.), SeqFold (Molecular Simulations Inc.), the MDL Available Chemicals Directory database, the MDL Drug Data Report data base, the Comprehensive Medicinal Chemistry database, Derwent's World Drug Index database, the BioByteMasterFile database, the Genbank database, and the Genseqn database. Many other programs and data bases would be apparent to one of skill in the art given the present disclosure.

Motifs which may be detected using the above programs include sequences encoding leucine zippers, helix-turn-helix motifs, glycosylation sites, ubiquitination sites, alpha helices, and beta sheets, signal sequences encoding signal peptides which direct the secretion of the encoded proteins, sequences implicated in transcription regulation such as homeoboxes, acidic stretches, enzymatic active sites, substrate binding sites, and enzymatic cleavage sites.

Hybridization of nucleic acids

The invention provides isolated or recombinant nucleic acids that hybridize under stringent conditions to an exemplary sequence of the invention, e.g., a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, or a nucleic acid that encodes a polypeptide comprising a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID

NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106. The stringent conditions can be highly stringent conditions, medium stringent conditions, low stringent conditions, including the high and reduced stringency conditions described herein. In alternative embodiments, nucleic acids of the invention as defined by their ability to hybridize under stringent conditions can be between about five residues and the full length of the molecule, e.g., an exemplary nucleic acid of the invention. For example, they can be at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 55, 60, 65, 70, 75, 80, 90, 100, 150, 200, 250, 300, 350, 400 residues in length. Nucleic acids shorter than full length are also included. These nucleic acids are useful as, e.g., hybridization probes, labeling probes, PCR oligonucleotide probes, iRNA (single or double stranded), antisense or sequences encoding antibody binding peptides (epitopes), motifs, active sites and the like.

In one aspect, nucleic acids of the invention are defined by their ability to hybridize under high stringency comprises conditions of about 50% formamide at about 37°C to 42°C. In one aspect, nucleic acids of the invention are defined by their ability to hybridize under reduced stringency comprising conditions in about 35% to 25% formamide at about 30°C to 35°C. Alternatively, nucleic acids of the invention are defined by their ability to hybridize under high stringency comprising conditions at 42°C in 50% formamide, 5X SSPE, 0.3% SDS, and a repetitive sequence blocking nucleic acid, such as cot-1 or salmon sperm DNA (e.g., 200 n/ml sheared and denatured salmon sperm DNA). In one aspect, nucleic acids of the invention are defined by their ability to hybridize under reduced stringency conditions comprising 35% formamide at a reduced temperature of 35°C.

Following hybridization, the filter may be washed with 6X SSC, 0.5% SDS at 50°C. These conditions are considered to be "moderate" conditions above 25% formamide and "low" conditions below 25% formamide. A specific example of "moderate" hybridization conditions is when the above hybridization is conducted at 30% formamide. A specific example of "low stringency" hybridization conditions is when the above hybridization is conducted at 10% formamide.

The temperature range corresponding to a particular level of stringency can be further narrowed by calculating the purine to pyrimidine ratio of the nucleic acid of interest and adjusting the temperature accordingly. Nucleic acids of the invention are also defined by their ability to hybridize under high, medium, and low stringency conditions as set forth in Ausubel and Sambrook. Variations on the above ranges and conditions are well known in the art. Hybridization conditions are discussed further, below.

Oligonucleotides probes and methods for using them

The invention also provides nucleic acid probes for identifying nucleic acids encoding a polypeptide with a phospholipase activity. In one aspect, the probe comprises at least 10 consecutive bases of a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105. Alternatively, a probe of the invention can be at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 50, 55, 60, 65, 70, 75, 80, 90, 100, 150, about 10 to 50, about 20 to 60 about 30 to 70, consecutive bases of a sequence as set forth in a sequence of the invention. The probes identify a nucleic acid by binding or hybridization. The probes can be used in arrays of the invention, see discussion below, including, e.g., capillary arrays. The probes of the invention can also be used to isolate other nucleic acids or polypeptides.

The probes of the invention can be used to determine whether a biological sample, such as a soil sample, contains an organism having a nucleic acid sequence of the invention or an organism from which the nucleic acid was obtained. In such procedures, a biological sample potentially harboring the organism from which the nucleic acid was isolated is obtained and nucleic acids are obtained from the sample. The nucleic acids are contacted with the probe under conditions which permit the probe to specifically hybridize to any complementary sequences present in the sample. Where necessary, conditions which permit the probe to specifically hybridize to complementary sequences may be determined by placing the probe in contact with complementary sequences from samples known to contain the complementary sequence, as well as control sequences which do not contain the complementary sequence. Hybridization conditions, such as the salt concentration of the hybridization buffer, the formamide concentration of the hybridization buffer, or the hybridization temperature, may be varied to identify conditions which allow the probe to

hybridize specifically to complementary nucleic acids (see discussion on specific hybridization conditions).

If the sample contains the organism from which the nucleic acid was isolated, specific hybridization of the probe is then detected. Hybridization may be detected by labeling the probe with a detectable agent such as a radioactive isotope, a fluorescent dye or an enzyme capable of catalyzing the formation of a detectable product. Many methods for using the labeled probes to detect the presence of complementary nucleic acids in a sample are familiar to those skilled in the art. These include Southern Blots, Northern Blots, colony hybridization procedures, and dot blots. Protocols for each of these procedures are provided in Ausubel and Sambrook.

Alternatively, more than one probe (at least one of which is capable of specifically hybridizing to any complementary sequences which are present in the nucleic acid sample), may be used in an amplification reaction to determine whether the sample contains an organism containing a nucleic acid sequence of the invention (e.g., an organism from which the nucleic acid was isolated). In one aspect, the probes comprise oligonucleotides. In one aspect, the amplification reaction may comprise a PCR reaction. PCR protocols are described in Ausubel and Sambrook (see discussion on amplification reactions). In such procedures, the nucleic acids in the sample are contacted with the probes, the amplification reaction is performed, and any resulting amplification product is detected. The amplification product may be detected by performing gel electrophoresis on the reaction products and staining the gel with an intercalator such as ethidium bromide. Alternatively, one or more of the probes may be labeled with a radioactive isotope and the presence of a radioactive amplification product may be detected by autoradiography after gel electrophoresis.

Probes derived from sequences near the 3' or 5' ends of a nucleic acid sequence of the invention can also be used in chromosome walking procedures to identify clones containing additional, e.g., genomic sequences. Such methods allow the isolation of genes which encode additional proteins of interest from the host organism.

In one aspect, nucleic acid sequences of the invention are used as probes to identify and isolate related nucleic acids. In some aspects, the so-identified related nucleic acids may be cDNAs or genomic DNAs from organisms other than the one from which the nucleic acid of the invention was first isolated. In such procedures, a nucleic acid sample is contacted with the probe under conditions which permit the probe to specifically hybridize to

related sequences. Hybridization of the probe to nucleic acids from the related organism is then detected using any of the methods described above.

In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition (e.g., GC v. AT content), and nucleic acid type (e.g., RNA v. DNA) of the hybridizing regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter. Hybridization may be carried out under conditions of low stringency, moderate stringency or high stringency. As an example of nucleic acid hybridization, a polymer membrane containing immobilized denatured nucleic acids is first prehybridized for 30 minutes at 45°C in a solution consisting of 0.9 M NaCl, 50 mM NaH2PO4, pH 7.0, 5.0 mM Na2EDTA, 0.5% SDS, 10X Denhardt's, and 0.5 mg/ml polyriboadenylic acid. Approximately 2 X 107 cpm (specific activity 4-9 X 108 cpm/ug) of ^{32}P end-labeled oligonucleotide probe are then added to the solution. After 12-16 hours of incubation, the membrane is washed for 30 minutes at room temperature (RT) in 1X SET (150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na2EDTA) containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at Tm-10°C for the oligonucleotide probe. The membrane is then exposed to auto-radiographic film for detection of hybridization signals.

By varying the stringency of the hybridization conditions used to identify nucleic acids, such as cDNAs or genomic DNAs, which hybridize to the detectable probe, nucleic acids having different levels of homology to the probe can be identified and isolated. Stringency may be varied by conducting the hybridization at varying temperatures below the melting temperatures of the probes. The melting temperature, Tm, is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly complementary probe. Very stringent conditions are selected to be equal to or about 5°C lower than the Tm for a particular probe. The melting temperature of the probe may be calculated using the following exemplary formulas. For probes between 14 and 70 nucleotides in length the melting temperature (Tm) is calculated using the formula: Tm=81.5+16.6(log [Na+])+0.41(fraction G+C)-(600/N) where N is the length of the probe. If the hybridization is carried out in a solution containing formamide, the melting temperature may be calculated using the equation: Tm=81.5+16.6(log [Na+])+0.41(fraction G+C)-(0.63% formamide)-(600/N) where N is the length of the probe. Prehybridization may be carried out

in 6X SSC, 5X Denhardt's reagent, 0.5% SDS, 100μg denatured fragmented salmon sperm DNA or 6X SSC, 5X Denhardt's reagent, 0.5% SDS, 100μg denatured fragmented salmon sperm DNA, 50% formamide. Formulas for SSC and Denhardt's and other solutions are listed, e.g., in Sambrook.

Hybridization is conducted by adding the detectable probe to the prehybridization solutions listed above. Where the probe comprises double stranded DNA, it is denatured before addition to the hybridization solution. The filter is contacted with the hybridization solution for a sufficient period of time to allow the probe to hybridize to cDNAs or genomic DNAs containing sequences complementary thereto or homologous thereto. For probes over 200 nucleotides in length, the hybridization may be carried out at 15-25°C below the Tm. For shorter probes, such as oligonucleotide probes, the hybridization may be conducted at 5-10°C below the Tm. In one aspect, hybridizations in 6X SSC are conducted at approximately 68°C. In one aspect, hybridizations in 50% formamide containing solutions are conducted at approximately 42°C. All of the foregoing hybridizations would be considered to be under conditions of high stringency.

Following hybridization, the filter is washed to remove any non-specifically bound detectable probe. The stringency used to wash the filters can also be varied depending on the nature of the nucleic acids being hybridized, the length of the nucleic acids being hybridized, the degree of complementarity, the nucleotide sequence composition (e.g., GC v. AT content), and the nucleic acid type (e.g., RNA v. DNA). Examples of progressively higher stringency condition washes are as follows: 2X SSC, 0.1% SDS at room temperature for 15 minutes (low stringency); 0.1X SSC, 0.5% SDS at room temperature for 30 minutes to 1 hour (moderate stringency); 0.1X SSC, 0.5% SDS for 15 to 30 minutes at between the hybridization temperature and 68°C (high stringency); and 0.15M NaCl for 15 minutes at 72°C (very high stringency). A final low stringency wash can be conducted in 0.1X SSC at room temperature. The examples above are merely illustrative of one set of conditions that can be used to wash filters. One of skill in the art would know that there are numerous recipes for different stringency washes.

Nucleic acids which have hybridized to the probe can be identified by autoradiography or other conventional techniques. The above procedure may be modified to identify nucleic acids having decreasing levels of homology to the probe sequence. For example, to obtain nucleic acids of decreasing homology to the detectable probe, less stringent conditions may be used. For example, the hybridization temperature may be decreased in increments of 5°C from 68°C to 42°C in a hybridization buffer having a Na+

concentration of approximately 1M. Following hybridization, the filter may be washed with 2X SSC, 0.5% SDS at the temperature of hybridization. These conditions are considered to be "moderate" conditions above 50°C and "low" conditions below 50°C. An example of "moderate" hybridization conditions is when the above hybridization is conducted at 55°C. An example of "low stringency" hybridization conditions is when the above hybridization is conducted at 45°C.

Alternatively, the hybridization may be carried out in buffers, such as 6X SSC, containing formamide at a temperature of 42°C. In this case, the concentration of formamide in the hybridization buffer may be reduced in 5% increments from 50% to 0% to identify clones having decreasing levels of homology to the probe. Following hybridization, the filter may be washed with 6X SSC, 0.5% SDS at 50°C. These conditions are considered to be "moderate" conditions above 25% formamide and "low" conditions below 25% formamide. A specific example of "moderate" hybridization conditions is when the above hybridization is conducted at 30% formamide. A specific example of "low stringency" hybridization conditions is when the above hybridization is conducted at 10% formamide.

These probes and methods of the invention can be used to isolate nucleic acids having a sequence with at least about 99%, 98%, 97%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, or at least 50% homology to a nucleic acid sequence of the invention comprising at least about 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 250, 300, 350, 400, or 500 consecutive bases thereof, and the sequences complementary thereto. Homology may be measured using an alignment algorithm, as discussed herein. For example, the homologous polynucleotides may have a coding sequence which is a naturally occurring allelic variant of one of the coding sequences described herein. Such allelic variants may have a substitution, deletion or addition of one or more nucleotides when compared to nucleic acids of the invention.

Additionally, the probes and methods of the invention may be used to isolate nucleic acids which encode polypeptides having at least about 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, or at least 50% sequence identity (homology) to a polypeptide of the invention comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof as determined using a sequence alignment algorithm (e.g., such as the FASTA version 3.0t78 algorithm with the default parameters, or a BLAST 2.2.2 program with exemplary settings as set forth herein).

Inhibiting Expression of Phospholipases

The invention further provides for nucleic acids complementary to (e.g., antisense sequences to) the nucleic acids of the invention, e.g., phospholipase-encoding nucleic acids. Antisense sequences are capable of inhibiting the transport, splicing or transcription of phospholipase-encoding genes. The inhibition can be effected through the targeting of genomic DNA or messenger RNA. The transcription or function of targeted nucleic acid can be inhibited, for example, by hybridization and/or cleavage. One particularly useful set of inhibitors provided by the present invention includes oligonucleotides which are able to either bind phospholipase gene or message, in either case preventing or inhibiting the production or function of phospholipase enzyme. The association can be though sequence specific hybridization. Another useful class of inhibitors includes oligonucleotides which cause inactivation or cleavage of phospholipase message. The oligonucleotide can have enzyme activity which causes such cleavage, such as ribozymes. The oligonucleotide can be chemically modified or conjugated to an enzyme or composition capable of cleaving the complementary nucleic acid. One may screen a pool of many different such oligonucleotides for those with the desired activity.

Inhibition of phospholipase expression can have a variety of industrial applications. For example, inhibition of phospholipase expression can slow or prevent spoilage. Spoilage can occur when lipids or polypeptides, e.g., structural lipids or polypeptides, are enzymatically degraded. This can lead to the deterioration, or rot, of fruits and vegetables. In one aspect, use of compositions of the invention that inhibit the expression and/or activity of phospholipase, e.g., antibodies, antisense oligonucleotides, ribozymes and RNAi, are used to slow or prevent spoilage. Thus, in one aspect, the invention provides methods and compositions comprising application onto a plant or plant product (e.g., a fruit, seed, root, leaf, etc.) antibodies, antisense oligonucleotides, ribozymes and RNAi of the invention to slow or prevent spoilage. These compositions also can be expressed by the plant (e.g., a transgenic plant) or another organism (e.g., a bacterium or other microorganism transformed with a phospholipase gene of the invention).

The compositions of the invention for the inhibition of phospholipase expression (e.g., antisense, iRNA, ribozymes, antibodies) can be used as pharmaceutical compositions.

Antisense Oligonucleotides

The invention provides antisense oligonucleotides capable of binding phospholipase message which can inhibit phospholipase activity by targeting mRNA. Strategies for designing antisense oligonucleotides are well described in the scientific and patent literature, and the skilled artisan can design such phospholipase oligonucleotides using the novel reagents of the invention. For example, gene walking/ RNA mapping protocols to screen for effective antisense oligonucleotides are well known in the art, see, e.g., Ho (2000) Methods Enzymol. 314:168-183, describing an RNA mapping assay, which is based on standard molecular techniques to provide an easy and reliable method for potent antisense sequence selection. See also Smith (2000) Eur. J. Pharm. Sci. 11:191-198.

Naturally occurring nucleic acids are used as antisense oligonucleotides. The antisense oligonucleotides can be of any length; for example, in alternative aspects, the antisense oligonucleotides are between about 5 to 100, about 10 to 80, about 15 to 60, about 18 to 40. The optimal length can be determined by routine screening. The antisense oligonucleotides can be present at any concentration. The optimal concentration can be determined by routine screening. A wide variety of synthetic, non-naturally occurring nucleotide and nucleic acid analogues are known which can address this potential problem. For example, peptide nucleic acids (PNAs) containing non-ionic backbones, such as N-(2-aminoethyl) glycine units can be used. Antisense oligonucleotides having phosphorothioate linkages can also be used, as described in WO 97/03211; WO 96/39154; Mata (1997) Toxicol Appl Pharmacol 144:189-197; Antisense Therapeutics, ed. Agrawal (Humana Press, Totowa, N.J., 1996). Antisense oligonucleotides having synthetic DNA backbone analogues provided by the invention can also include phosphoro-dithioate, methylphosphonate, phosphoramidate, alkyl phosphotriester, sulfamate, 3'-thioacetal, methylene(methylimino), 3'-N-carbamate, and morpholino carbamate nucleic acids, as described above.

Combinatorial chemistry methodology can be used to create vast numbers of oligonucleotides that can be rapidly screened for specific oligonucleotides that have appropriate binding affinities and specificities toward any target, such as the sense and antisense phospholipase sequences of the invention (see, e.g., Gold (1995) J. of Biol. Chem. 270:13581-13584).

Inhibitory Ribozymes

The invention provides for with ribozymes capable of binding phospholipase message which can inhibit phospholipase enzyme activity by targeting mRNA. Strategies for designing ribozymes and selecting the phospholipase-specific antisense sequence for

targeting are well described in the scientific and patent literature, and the skilled artisan can design such ribozymes using the novel reagents of the invention. Ribozymes act by binding to a target RNA through the target RNA binding portion of a ribozyme which is held in close proximity to an enzymatic portion of the RNA that cleaves the target RNA. Thus, the ribozyme recognizes and binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cleave and inactivate the target RNA. Cleavage of a target RNA in such a manner will destroy its ability to direct synthesis of an encoded protein if the cleavage occurs in the coding sequence. After a ribozyme has bound and cleaved its RNA target, it is typically released from that RNA and so can bind and cleave new targets repeatedly.

In some circumstances, the enzymatic nature of a ribozyme can be advantageous over other technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its transcription, translation or association with another molecule) as the effective concentration of ribozyme necessary to effect a therapeutic treatment can be lower than that of an antisense oligonucleotide. This potential advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, a ribozyme is typically a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding, but also on the mechanism by which the molecule inhibits the expression of the RNA to which it binds. That is, the inhibition is caused by cleavage of the RNA target and so specificity is defined as the ratio of the rate of cleavage of the targeted RNA over the rate of cleavage of non-targeted RNA. This cleavage mechanism is dependent upon factors additional to those involved in base pairing. Thus, the specificity of action of a ribozyme can be greater than that of antisense oligonucleotide binding the same RNA site.

The enzymatic ribozyme RNA molecule can be formed in a hammerhead motif, but may also be formed in the motif of a hairpin, hepatitis delta virus, group I intron or RNaseP-like RNA (in association with an RNA guide sequence). Examples of such hammerhead motifs are described by Rossi (1992) Aids Research and Human Retroviruses 8:183; hairpin motifs by Hampel (1989) Biochemistry 28:4929, and Hampel (1990) Nuc. Acids Res. 18:299; the hepatitis delta virus motif by Perrotta (1992) Biochemistry 31:16; the RNaseP motif by Guerrier-Takada (1983) Cell 35:849; and the group I intron by Cech U.S. Pat. No. 4,987,071. The recitation of these specific motifs is not intended to be limiting; those skilled in the art will recognize that an enzymatic RNA molecule of this invention has a

specific substrate binding site complementary to one or more of the target gene RNA regions, and has nucleotide sequence within or surrounding that substrate binding site which imparts an RNA cleaving activity to the molecule.

RNA interference (RNAi)

In one aspect, the invention provides an RNA inhibitory molecule, a so-called "RNAi" molecule, comprising a phospholipase sequence of the invention. The RNAi molecule comprises a double-stranded RNA (dsRNA) molecule. The RNAi can inhibit expression of a phospholipase gene. In one aspect, the RNAi is about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more duplex nucleotides in length. While the invention is not limited by any particular mechanism of action, the RNAi can enter a cell and cause the degradation of a single-stranded RNA (ssRNA) of similar or identical sequences, including endogenous mRNAs. When a cell is exposed to double-stranded RNA (dsRNA), mRNA from the homologous gene is selectively degraded by a process called RNA interference (RNAi). A possible basic mechanism behind RNAi is the breaking of a double-stranded RNA (dsRNA) matching a specific gene sequence into short pieces called short interfering RNA, which trigger the degradation of mRNA that matches its sequence. In one aspect, the RNAi's of the invention are used in gene-silencing therapeutics, see, e.g., Shuey (2002) Drug Discov. Today 7:1040-1046. In one aspect, the invention provides methods to selectively degrade RNA using the RNAi's of the invention. The process may be practiced *in vitro*, *ex vivo* or *in vivo*. In one aspect, the RNAi molecules of the invention can be used to generate a loss-of-function mutation in a cell, an organ or an animal. Methods for making and using RNAi molecules for selectively degrade RNA are well known in the art, see, e.g., U.S. Patent No. 6,506,559; 6,511,824; 6,515,109; 6,489,127.

Modification of Nucleic Acids

The invention provides methods of generating variants of the nucleic acids of the invention, e.g., those encoding a phospholipase enzyme. These methods can be repeated or used in various combinations to generate phospholipase enzymes having an altered or different activity or an altered or different stability from that of a phospholipase encoded by the template nucleic acid. These methods also can be repeated or used in various combinations, e.g., to generate variations in gene/ message expression, message translation or message stability. In another aspect, the genetic composition of a cell is altered by, e.g., modification of a homologous gene *ex vivo*, followed by its reinsertion into the cell.

A nucleic acid of the invention can be altered by any means. For example, random or stochastic methods, or, non-stochastic, or "directed evolution," methods.

Methods for random mutation of genes are well known in the art, see, e.g., U.S. Patent No. 5,830,696. For example, mutagens can be used to randomly mutate a gene. Mutagens include, e.g., ultraviolet light or gamma irradiation, or a chemical mutagen, e.g., mitomycin, nitrous acid, photoactivated psoralens, alone or in combination, to induce DNA breaks amenable to repair by recombination. Other chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other mutagens are analogues of nucleotide precursors, e.g., nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. These agents can be added to a PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used.

Any technique in molecular biology can be used, e.g., random PCR mutagenesis, see, e.g., Rice (1992) Proc. Natl. Acad. Sci. USA 89:5467-5471; or, combinatorial multiple cassette mutagenesis, see, e.g., Crameri (1995) Biotechniques 18:194-196. Alternatively, nucleic acids, e.g., genes, can be reassembled after random, or "stochastic," fragmentation, see, e.g., U.S. Patent Nos. 6,291,242; 6,287,862; 6,287,861; 5,955,358; 5,830,721; 5,824,514; 5,811,238; 5,605,793. In alternative aspects, modifications, additions or deletions are introduced by error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSM), synthetic ligation reassembly (SLR), recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation, and/or a combination of these and other methods.

The following publications describe a variety of recursive recombination procedures and/or methods which can be incorporated into the methods of the invention: Stemmer (1999) "Molecular breeding of viruses for targeting and other clinical properties" Tumor Targeting 4:1-4; Ness (1999) Nature Biotechnology 17:893-896; Chang (1999) "Evolution of a cytokine using DNA family shuffling" Nature Biotechnology 17:793-797;

Minshull (1999) "Protein evolution by molecular breeding" Current Opinion in Chemical Biology 3:284-290; Christians (1999) "Directed evolution of thymidine kinase for AZT phosphorylation using DNA family shuffling" Nature Biotechnology 17:259-264; Crameri (1998) "DNA shuffling of a family of genes from diverse species accelerates directed evolution" Nature 391:288-291; Crameri (1997) "Molecular evolution of an arsenate detoxification pathway by DNA shuffling," Nature Biotechnology 15:436-438; Zhang (1997) "Directed evolution of an effective fucosidase from a galactosidase by DNA shuffling and screening" Proc. Natl. Acad. Sci. USA 94:4504-4509; Patten et al. (1997) "Applications of DNA Shuffling to Pharmaceuticals and Vaccines" Current Opinion in Biotechnology 8:724-733; Crameri et al. (1996) "Construction and evolution of antibody-phage libraries by DNA shuffling" Nature Medicine 2:100-103; Crameri et al. (1996) "Improved green fluorescent protein by molecular evolution using DNA shuffling" Nature Biotechnology 14:315-319; Gates et al. (1996) "Affinity selective isolation of ligands from peptide libraries through display on a lac repressor `headpiece dimer`" Journal of Molecular Biology 255:373-386; Stemmer (1996) "Sexual PCR and Assembly PCR" In: The Encyclopedia of Molecular Biology. VCH Publishers, New York. pp.447-457; Crameri and Stemmer (1995) "Combinatorial multiple cassette mutagenesis creates all the permutations of mutant and wildtype cassettes" BioTechniques 18:194-195; Stemmer et al. (1995) "Single-step assembly of a gene and entire plasmid form large numbers of oligodeoxyribonucleotides" Gene, 164:49-53; Stemmer (1995) "The Evolution of Molecular Computation" Science 270: 1510; Stemmer (1995) "Searching Sequence Space" Bio/Technology 13:549-553; Stemmer (1994) "Rapid evolution of a protein in vitro by DNA shuffling" Nature 370:389-391; and Stemmer (1994) "DNA shuffling by random fragmentation and reassembly: In vitro recombination for molecular evolution." Proc. Natl. Acad. Sci. USA 91:10747-10751.

Mutational methods of generating diversity include, for example, site-directed mutagenesis (Ling et al. (1997) "Approaches to DNA mutagenesis: an overview" Anal Biochem. 254(2): 157-178; Dale et al. (1996) "Oligonucleotide-directed random mutagenesis using the phosphorothioate method" Methods Mol. Biol. 57:369-374; Smith (1985) "In vitro mutagenesis" Ann. Rev. Genet. 19:423-462; Botstein & Shortle (1985) "Strategies and applications of in vitro mutagenesis" Science 229:1193-1201; Carter (1986) "Site-directed mutagenesis" Biochem. J. 237:1-7; and Kunkel (1987) "The efficiency of oligonucleotide directed mutagenesis" in Nucleic Acids & Molecular Biology (Eckstein, F. and Lilley, D. M. J. eds., Springer Verlag, Berlin)); mutagenesis using uracil containing templates (Kunkel (1985) "Rapid and efficient site-specific mutagenesis without phenotypic selection" Proc.

Natl. Acad. Sci. USA 82:488-492; Kunkel et al. (1987) "Rapid and efficient site-specific mutagenesis without phenotypic selection" Methods in Enzymol. 154, 367-382; and Bass et al. (1988) "Mutant Trp repressors with new DNA-binding specificities" Science 242:240-245); oligonucleotide-directed mutagenesis (Methods in Enzymol. 100: 468-500 (1983); Methods in Enzymol. 154: 329-350 (1987); Zoller & Smith (1982) "Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any DNA fragment" Nucleic Acids Res. 10:6487-6500; Zoller & Smith (1983) "Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors" Methods in Enzymol. 100:468-500; and Zoller & Smith (1987) "Oligonucleotide-directed mutagenesis: a simple method using two oligonucleotide primers and a single-stranded DNA template" Methods in Enzymol. 154:329-350); phosphorothioate-modified DNA mutagenesis (Taylor et al. (1985) "The use of phosphorothioate-modified DNA in restriction enzyme reactions to prepare nicked DNA" Nucl. Acids Res. 13: 8749-8764; Taylor et al. (1985) "The rapid generation of oligonucleotide-directed mutations at high frequency using phosphorothioate-modified DNA" Nucl. Acids Res. 13: 8765-8787 (1985); Nakamaye (1986) "Inhibition of restriction endonuclease Nci I cleavage by phosphorothioate groups and its application to oligonucleotide-directed mutagenesis" Nucl. Acids Res. 14: 9679-9698; Sayers et al. (1988) "Y-T Exonucleases in phosphorothioate-based oligonucleotide-directed mutagenesis" Nucl. Acids Res. 16:791-802; and Sayers et al. (1988) "Strand specific cleavage of phosphorothioate-containing DNA by reaction with restriction endonucleases in the presence of ethidium bromide" Nucl. Acids Res. 16: 803-814); mutagenesis using gapped duplex DNA (Kramer et al. (1984) "The gapped duplex DNA approach to oligonucleotide-directed mutation construction" Nucl. Acids Res. 12: 9441-9456; Kramer & Fritz (1987) Methods in Enzymol. "Oligonucleotide-directed construction of mutations via gapped duplex DNA" 154:350-367; Kramer et al. (1988) "Improved enzymatic in vitro reactions in the gapped duplex DNA approach to oligonucleotide-directed construction of mutations" Nucl. Acids Res. 16: 7207; and Fritz et al. (1988) "Oligonucleotide-directed construction of mutations: a gapped duplex DNA procedure without enzymatic reactions in vitro" Nucl. Acids Res. 16: 6987-6999).

Additional protocols used in the methods of the invention include point mismatch repair (Kramer (1984) "Point Mismatch Repair" Cell 38:879-887), mutagenesis using repair-deficient host strains (Carter et al. (1985) "Improved oligonucleotide site-directed mutagenesis using M13 vectors" Nucl. Acids Res. 13: 4431-4443; and Carter (1987) "Improved oligonucleotide-directed mutagenesis using M13 vectors" Methods in Enzymol.

154: 382-403), deletion mutagenesis (Eghtedarzadeh (1986) "Use of oligonucleotides to generate large deletions" Nucl. Acids Res. 14: 5115), restriction-selection and restriction-selection and restriction-purification (Wells et al. (1986) "Importance of hydrogen-bond formation in stabilizing the transition state of subtilisin" Phil. Trans. R. Soc. Lond. A 317: 415-423), mutagenesis by total gene synthesis (Nambiar et al. (1984) "Total synthesis and cloning of a gene coding for the ribonuclease S protein" Science 223: 1299-1301; Sakamar and Khorana (1988) "Total synthesis and expression of a gene for the a-subunit of bovine rod outer segment guanine nucleotide-binding protein (transducin)" Nucl. Acids Res. 14: 6361-6372; Wells et al. (1985) "Cassette mutagenesis: an efficient method for generation of multiple mutations at defined sites" Gene 34:315-323; and Grundstrom et al. (1985) "Oligonucleotide-directed mutagenesis by microscale 'shot-gun' gene synthesis" Nucl. Acids Res. 13: 3305-3316), double-strand break repair (Mandecki (1986); Arnold (1993) "Protein engineering for unusual environments" Current Opinion in Biotechnology 4:450-455. "Oligonucleotide-directed double-strand break repair in plasmids of Escherichia coli: a method for site-specific mutagenesis" Proc. Natl. Acad. Sci. USA, 83:7177-7181). Additional details on many of the above methods can be found in Methods in Enzymology Volume 154, which also describes useful controls for trouble-shooting problems with various mutagenesis methods.

See also U.S. Patent Nos. 5,605,793 to Stemmer (Feb. 25, 1997), "Methods for In Vitro Recombination;" U.S. Pat. No. 5,811,238 to Stemmer et al. (Sep. 22, 1998) "Methods for Generating Polynucleotides having Desired Characteristics by Iterative Selection and Recombination;" U.S. Pat. No. 5,830,721 to Stemmer et al. (Nov. 3, 1998), "DNA Mutagenesis by Random Fragmentation and Reassembly;" U.S. Pat. No. 5,834,252 to Stemmer, et al. (Nov. 10, 1998) "End-Complementary Polymerase Reaction;" U.S. Pat. No. 5,837,458 to Minshull, et al. (Nov. 17, 1998), "Methods and Compositions for Cellular and Metabolic Engineering;" WO 95/22625, Stemmer and Crameri, "Mutagenesis by Random Fragmentation and Reassembly;" WO 96/33207 by Stemmer and Lipschutz "End Complementary Polymerase Chain Reaction;" WO 97/20078 by Stemmer and Crameri "Methods for Generating Polynucleotides having Desired Characteristics by Iterative Selection and Recombination;" WO 97/35966 by Minshull and Stemmer, "Methods and Compositions for Cellular and Metabolic Engineering;" WO 99/41402 by Punnonen et al. "Targeting of Genetic Vaccine Vectors;" WO 99/41383 by Punnonen et al. "Antigen Library Immunization;" WO 99/41369 by Punnonen et al. "Genetic Vaccine Vector Engineering;" WO 99/41368 by Punnonen et al. "Optimization of Immunomodulatory Properties of Genetic

Vaccines;" EP 752008 by Stemmer and Crameri, "DNA Mutagenesis by Random Fragmentation and Reassembly;" EP 0932670 by Stemmer "Evolving Cellular DNA Uptake by Recursive Sequence Recombination;" WO 99/23107 by Stemmer et al., "Modification of Virus Tropism and Host Range by Viral Genome Shuffling;" WO 99/21979 by Apt et al., "Human Papillomavirus Vectors;" WO 98/31837 by del Cardayre et al. "Evolution of Whole Cells and Organisms by Recursive Sequence Recombination;" WO 98/27230 by Patten and Stemmer, "Methods and Compositions for Polypeptide Engineering;" WO 98/27230 by Stemmer et al., "Methods for Optimization of Gene Therapy by Recursive Sequence Shuffling and Selection," WO 00/00632, "Methods for Generating Highly Diverse Libraries," WO 00/09679, "Methods for Obtaining in Vitro Recombined Polynucleotide Sequence Banks and Resulting Sequences," WO 98/42832 by Arnold et al., "Recombination of Polynucleotide Sequences Using Random or Defined Primers," WO 99/29902 by Arnold et al., "Method for Creating Polynucleotide and Polypeptide Sequences," WO 98/41653 by Vind, "An in Vitro Method for Construction of a DNA Library," WO 98/41622 by Borchert et al., "Method for Constructing a Library Using DNA Shuffling," and WO 98/42727 by Pati and Zarling, "Sequence Alterations using Homologous Recombination."

Certain U.S. applications provide additional details regarding various diversity generating methods, including "SHUFFLING OF CODON ALTERED GENES" by Patten et al. filed Sep. 28, 1999, (U.S. Ser. No. 09/407,800); "EVOLUTION OF WHOLE CELLS AND ORGANISMS BY RECURSIVE SEQUENCE RECOMBINATION" by del Cardayre et al., filed Jul. 15, 1998 (U.S. Ser. No. 09/166,188), and Jul. 15, 1999 (U.S. Ser. No. 09/354,922); "OLIGONUCLEOTIDE MEDIATED NUCLEIC ACID RECOMBINATION" by Crameri et al., filed Sep. 28, 1999 (U.S. Ser. No. 09/408,392), and "OLIGONUCLEOTIDE MEDIATED NUCLEIC ACID RECOMBINATION" by Crameri et al., filed Jan. 18, 2000 (PCT/US00/01203); "USE OF CODON-VARIED OLIGONUCLEOTIDE SYNTHESIS FOR SYNTHETIC SHUFFLING" by Welch et al., filed Sep. 28, 1999 (U.S. Ser. No. 09/408,393); "METHODS FOR MAKING CHARACTER STRINGS, POLYNUCLEOTIDES & POLYPEPTIDES HAVING DESIRED CHARACTERISTICS" by Selifonov et al., filed Jan. 18, 2000, (PCT/US00/01202) and, e.g. "METHODS FOR MAKING CHARACTER STRINGS, POLYNUCLEOTIDES & POLYPEPTIDES HAVING DESIRED CHARACTERISTICS" by Selifonov et al., filed Jul. 18, 2000 (U.S. Ser. No. 09/618,579); "METHODS OF POPULATING DATA STRUCTURES FOR USE IN EVOLUTIONARY SIMULATIONS" by Selifonov and Stemmer, filed Jan. 18, 2000 (PCT/US00/01138); and "SINGLE-STRANDED NUCLEIC

ACID TEMPLATE-MEDIATED RECOMBINATION AND NUCLEIC ACID FRAGMENT ISOLATION" by Affholter, filed Sep. 6, 2000 (U.S. Ser. No. 09/656,549).

Non-stochastic, or "directed evolution," methods include, e.g., saturation mutagenesis (GSSM), synthetic ligation reassembly (SLR), or a combination thereof are used to modify the nucleic acids of the invention to generate phospholipases with new or altered properties (e.g., activity under highly acidic or alkaline conditions, high temperatures, and the like). Polypeptides encoded by the modified nucleic acids can be screened for an activity before testing for an phospholipase or other activity. Any testing modality or protocol can be used, e.g., using a capillary array platform. See, e.g., U.S. Patent Nos. 6,280,926; 5,939,250.

Saturation mutagenesis, or, GSSM

In one aspect of the invention, non-stochastic gene modification, a "directed evolution process," is used to generate phospholipases with new or altered properties. Variations of this method have been termed "gene site-saturation mutagenesis," "site-saturation mutagenesis," "saturation mutagenesis" or simply "GSSM." It can be used in combination with other mutagenization processes. See, e.g., U.S. Patent Nos. 6,171,820; 6,238,884. In one aspect, GSSM comprises providing a template polynucleotide and a plurality of oligonucleotides, wherein each oligonucleotide comprises a sequence homologous to the template polynucleotide, thereby targeting a specific sequence of the template polynucleotide, and a sequence that is a variant of the homologous gene; generating progeny polynucleotides comprising non-stochastic sequence variations by replicating the template polynucleotide with the oligonucleotides, thereby generating polynucleotides comprising homologous gene sequence variations.

In one aspect, codon primers containing a degenerate N,N,G/T sequence are used to introduce point mutations into a polynucleotide, so as to generate a set of progeny polypeptides in which a full range of single amino acid substitutions is represented at each amino acid position, e.g., an amino acid residue in an enzyme active site or ligand binding site targeted to be modified. These oligonucleotides can comprise a contiguous first homologous sequence, a degenerate N,N,G/T sequence, and, optionally, a second homologous sequence. The downstream progeny translational products from the use of such oligonucleotides include all possible amino acid changes at each amino acid site along the polypeptide, because the degeneracy of the N,N,G/T sequence includes codons for all 20 amino acids. In one aspect, one such degenerate oligonucleotide (comprised of, e.g., one degenerate N,N,G/T cassette) is used for subjecting each original codon in a parental

polynucleotide template to a full range of codon substitutions. In another aspect, at least two degenerate cassettes are used – either in the same oligonucleotide or not, for subjecting at least two original codons in a parental polynucleotide template to a full range of codon substitutions. For example, more than one N,N,G/T sequence can be contained in one oligonucleotide to introduce amino acid mutations at more than one site. This plurality of N,N,G/T sequences can be directly contiguous, or separated by one or more additional nucleotide sequence(s). In another aspect, oligonucleotides serviceable for introducing additions and deletions can be used either alone or in combination with the codons containing an N,N,G/T sequence, to introduce any combination or permutation of amino acid additions, deletions, and/or substitutions.

In one aspect, simultaneous mutagenesis of two or more contiguous amino acid positions is done using an oligonucleotide that contains contiguous N,N,G/T triplets, i.e. a degenerate (N,N,G/T)n sequence. In another aspect, degenerate cassettes having less degeneracy than the N,N,G/T sequence are used. For example, it may be desirable in some instances to use (e.g. in an oligonucleotide) a degenerate triplet sequence comprised of only one N, where said N can be in the first second or third position of the triplet. Any other bases including any combinations and permutations thereof can be used in the remaining two positions of the triplet. Alternatively, it may be desirable in some instances to use (e.g. in an oligo) a degenerate N,N,N triplet sequence.

In one aspect, use of degenerate triplets (e.g., N,N,G/T triplets) allows for systematic and easy generation of a full range of possible natural amino acids (for a total of 20 amino acids) into each and every amino acid position in a polypeptide (in alternative aspects, the methods also include generation of less than all possible substitutions per amino acid residue, or codon, position). For example, for a 100 amino acid polypeptide, 2000 distinct species (i.e. 20 possible amino acids per position X 100 amino acid positions) can be generated. Through the use of an oligonucleotide or set of oligonucleotides containing a degenerate N,N,G/T triplet, 32 individual sequences can code for all 20 possible natural amino acids. Thus, in a reaction vessel in which a parental polynucleotide sequence is subjected to saturation mutagenesis using at least one such oligonucleotide, there are generated 32 distinct progeny polynucleotides encoding 20 distinct polypeptides. In contrast, the use of a non-degenerate oligonucleotide in site-directed mutagenesis leads to only one progeny polypeptide product per reaction vessel. Nondegenerate oligonucleotides can optionally be used in combination with degenerate primers disclosed; for example, nondegenerate oligonucleotides can be used to generate specific point mutations in a working

polynucleotide. This provides one means to generate specific silent point mutations, point mutations leading to corresponding amino acid changes, and point mutations that cause the generation of stop codons and the corresponding expression of polypeptide fragments.

In one aspect, each saturation mutagenesis reaction vessel contains polynucleotides encoding at least 20 progeny polypeptide (e.g., phospholipase) molecules such that all 20 natural amino acids are represented at the one specific amino acid position corresponding to the codon position mutagenized in the parental polynucleotide (other aspects use less than all 20 natural combinations). The 32-fold degenerate progeny polypeptides generated from each saturation mutagenesis reaction vessel can be subjected to clonal amplification (e.g. cloned into a suitable host, e.g., *E. coli* host, using, e.g., an expression vector) and subjected to expression screening. When an individual progeny polypeptide is identified by screening to display a favorable change in property (when compared to the parental polypeptide, such as increased phospholipase activity under alkaline or acidic conditions), it can be sequenced to identify the correspondingly favorable amino acid substitution contained therein.

In one aspect, upon mutagenizing each and every amino acid position in a parental polypeptide using saturation mutagenesis as disclosed herein, favorable amino acid changes may be identified at more than one amino acid position. One or more new progeny molecules can be generated that contain a combination of all or part of these favorable amino acid substitutions. For example, if 2 specific favorable amino acid changes are identified in each of 3 amino acid positions in a polypeptide, the permutations include 3 possibilities at each position (no change from the original amino acid, and each of two favorable changes) and 3 positions. Thus, there are 3 x 3 x 3 or 27 total possibilities, including 7 that were previously examined - 6 single point mutations (i.e. 2 at each of three positions) and no change at any position.

In another aspect, site-saturation mutagenesis can be used together with another stochastic or non-stochastic means to vary sequence, e.g., synthetic ligation reassembly (see below), shuffling, chimerization, recombination and other mutagenizing processes and mutagenizing agents. This invention provides for the use of any mutagenizing process(es), including saturation mutagenesis, in an iterative manner.

Synthetic Ligation Reassembly (SLR)

The invention provides a non-stochastic gene modification system termed "synthetic ligation reassembly," or simply "SLR," a "directed evolution process," to generate

phospholipases with new or altered properties. SLR is a method of ligating oligonucleotide fragments together non-stochastically. This method differs from stochastic oligonucleotide shuffling in that the nucleic acid building blocks are not shuffled, concatenated or chimerized randomly, but rather are assembled non-stochastically. See, e.g., U.S. Patent Application Serial No. (USSN) 09/332,835 entitled "Synthetic Ligation Reassembly in Directed Evolution" and filed on June 14, 1999 ("USSN 09/332,835"). In one aspect, SLR comprises the following steps: (a) providing a template polynucleotide, wherein the template polynucleotide comprises sequence encoding a homologous gene; (b) providing a plurality of building block polynucleotides, wherein the building block polynucleotides are designed to cross-over reassemble with the template polynucleotide at a predetermined sequence, and a building block polynucleotide comprises a sequence that is a variant of the homologous gene and a sequence homologous to the template polynucleotide flanking the variant sequence; (c) combining a building block polynucleotide with a template polynucleotide such that the building block polynucleotide cross-over reassembles with the template polynucleotide to generate polynucleotides comprising homologous gene sequence variations.

SLR does not depend on the presence of high levels of homology between polynucleotides to be rearranged. Thus, this method can be used to non-stochastically generate libraries (or sets) of progeny molecules comprised of over 10^{100} different chimeras. SLR can be used to generate libraries comprised of over 10^{1000} different progeny chimeras. Thus, aspects of the present invention include non-stochastic methods of producing a set of finalized chimeric nucleic acid molecule shaving an overall assembly order that is chosen by design. This method includes the steps of generating by design a plurality of specific nucleic acid building blocks having serviceable mutually compatible ligatable ends, and assembling these nucleic acid building blocks, such that a designed overall assembly order is achieved.

The mutually compatible ligatable ends of the nucleic acid building blocks to be assembled are considered to be "serviceable" for this type of ordered assembly if they enable the building blocks to be coupled in predetermined orders. Thus the overall assembly order in which the nucleic acid building blocks can be coupled is specified by the design of the ligatable ends. If more than one assembly step is to be used, then the overall assembly order in which the nucleic acid building blocks can be coupled is also specified by the sequential order of the assembly step(s). In one aspect, the annealed building pieces are treated with an enzyme, such as a ligase (e.g. T4 DNA ligase), to achieve covalent bonding of the building pieces.

In one aspect, the design of the oligonucleotide building blocks is obtained by analyzing a set of progenitor nucleic acid sequence templates that serve as a basis for producing a progeny set of finalized chimeric polynucleotides. These parental oligonucleotide templates thus serve as a source of sequence information that aids in the design of the nucleic acid building blocks that are to be mutagenized, e.g., chimerized or shuffled.

In one aspect of this method, the sequences of a plurality of parental nucleic acid templates are aligned in order to select one or more demarcation points. The demarcation points can be located at an area of homology, and are comprised of one or more nucleotides. These demarcation points are preferably shared by at least two of the progenitor templates. The demarcation points can thereby be used to delineate the boundaries of oligonucleotide building blocks to be generated in order to rearrange the parental polynucleotides. The demarcation points identified and selected in the progenitor molecules serve as potential chimerization points in the assembly of the final chimeric progeny molecules. A demarcation point can be an area of homology (comprised of at least one homologous nucleotide base) shared by at least two parental polynucleotide sequences. Alternatively, a demarcation point can be an area of homology that is shared by at least half of the parental polynucleotide sequences, or, it can be an area of homology that is shared by at least two thirds of the parental polynucleotide sequences. Even more preferably a serviceable demarcation points is an area of homology that is shared by at least three fourths of the parental polynucleotide sequences, or, it can be shared by at almost all of the parental polynucleotide sequences. In one aspect, a demarcation point is an area of homology that is shared by all of the parental polynucleotide sequences.

In one aspect, a ligation reassembly process is performed exhaustively in order to generate an exhaustive library of progeny chimeric polynucleotides. In other words, all possible ordered combinations of the nucleic acid building blocks are represented in the set of finalized chimeric nucleic acid molecules. At the same time, in another embodiment, the assembly order (i.e. the order of assembly of each building block in the 5' to 3 sequence of each finalized chimeric nucleic acid) in each combination is by design (or non-stochastic) as described above. Because of the non-stochastic nature of this invention, the possibility of unwanted side products is greatly reduced.

In another aspect, the ligation reassembly method is performed systematically. For example, the method is performed in order to generate a systematically compartmentalized library of progeny molecules, with compartments that can be screened

systematically, e.g. one by one. In other words this invention provides that, through the selective and judicious use of specific nucleic acid building blocks, coupled with the selective and judicious use of sequentially stepped assembly reactions, a design can be achieved where specific sets of progeny products are made in each of several reaction vessels. This allows a systematic examination and screening procedure to be performed. Thus, these methods allow a potentially very large number of progeny molecules to be examined systematically in smaller groups. Because of its ability to perform chimerizations in a manner that is highly flexible yet exhaustive and systematic as well, particularly when there is a low level of homology among the progenitor molecules, these methods provide for the generation of a library (or set) comprised of a large number of progeny molecules. Because of the non-stochastic nature of the instant ligation reassembly invention, the progeny molecules generated preferably comprise a library of finalized chimeric nucleic acid molecules having an overall assembly order that is chosen by design. The saturation mutagenesis and optimized directed evolution methods also can be used to generate different progeny molecular species. It is appreciated that the invention provides freedom of choice and control regarding the selection of demarcation points, the size and number of the nucleic acid building blocks, and the size and design of the couplings. It is appreciated, furthermore, that the requirement for intermolecular homology is highly relaxed for the operability of this invention. In fact, demarcation points can even be chosen in areas of little or no intermolecular homology. For example, because of codon wobble, i.e. the degeneracy of codons, nucleotide substitutions can be introduced into nucleic acid building blocks without altering the amino acid originally encoded in the corresponding progenitor template. Alternatively, a codon can be altered such that the coding for an originally amino acid is altered. This invention provides that such substitutions can be introduced into the nucleic acid building block in order to increase the incidence of intermolecularly homologous demarcation points and thus to allow an increased number of couplings to be achieved among the building blocks, which in turn allows a greater number of progeny chimeric molecules to be generated.

In another aspect, the synthetic nature of the step in which the building blocks are generated allows the design and introduction of nucleotides (e.g., one or more nucleotides, which may be, for example, codons or introns or regulatory sequences) that can later be optionally removed in an *in vitro* process (e.g. by mutagenesis) or in an *in vivo* process (e.g. by utilizing the gene splicing ability of a host organism). It is appreciated that in

many instances the introduction of these nucleotides may also be desirable for many other reasons in addition to the potential benefit of creating a serviceable demarcation point.

In one aspect, a nucleic acid building block is used to introduce an intron. Thus, functional introns are introduced into a man-made gene manufactured according to the methods described herein. The artificially introduced intron(s) can be functional in a host cells for gene splicing much in the way that naturally-occurring introns serve functionally in gene splicing.

Optimized Directed Evolution System

The invention provides a non-stochastic gene modification system termed "optimized directed evolution system" to generate phospholipases with new or altered properties. Optimized directed evolution is directed to the use of repeated cycles of reductive reassortment, recombination and selection that allow for the directed molecular evolution of nucleic acids through recombination. Optimized directed evolution allows generation of a large population of evolved chimeric sequences, wherein the generated population is significantly enriched for sequences that have a predetermined number of crossover events.

A crossover event is a point in a chimeric sequence where a shift in sequence occurs from one parental variant to another parental variant. Such a point is normally at the juncture of where oligonucleotides from two parents are ligated together to form a single sequence. This method allows calculation of the correct concentrations of oligonucleotide sequences so that the final chimeric population of sequences is enriched for the chosen number of crossover events. This provides more control over choosing chimeric variants having a predetermined number of crossover events.

In addition, this method provides a convenient means for exploring a tremendous amount of the possible protein variant space in comparison to other systems. Previously, if one generated, for example, 10^{13} chimeric molecules during a reaction, it would be extremely difficult to test such a high number of chimeric variants for a particular activity. Moreover, a significant portion of the progeny population would have a very high number of crossover events which resulted in proteins that were less likely to have increased levels of a particular activity. By using these methods, the population of chimerics molecules can be enriched for those variants that have a particular number of crossover events. Thus, although one can still generate 10^{13} chimeric molecules during a reaction, each of the molecules chosen for further analysis most likely has, for example, only three crossover events. Because the resulting progeny population can be skewed to have a predetermined number of

crossover events, the boundaries on the functional variety between the chimeric molecules is reduced. This provides a more manageable number of variables when calculating which oligonucleotide from the original parental polynucleotides might be responsible for affecting a particular trait.

One method for creating a chimeric progeny polynucleotide sequence is to create oligonucleotides corresponding to fragments or portions of each parental sequence. Each oligonucleotide preferably includes a unique region of overlap so that mixing the oligonucleotides together results in a new variant that has each oligonucleotide fragment assembled in the correct order. Additional information can also be found in USSN 09/332,835. The number of oligonucleotides generated for each parental variant bears a relationship to the total number of resulting crossovers in the chimeric molecule that is ultimately created. For example, three parental nucleotide sequence variants might be provided to undergo a ligation reaction in order to find a chimeric variant having, for example, greater activity at high temperature. As one example, a set of 50 oligonucleotide sequences can be generated corresponding to each portions of each parental variant. Accordingly, during the ligation reassembly process there could be up to 50 crossover events within each of the chimeric sequences. The probability that each of the generated chimeric polynucleotides will contain oligonucleotides from each parental variant in alternating order is very low. If each oligonucleotide fragment is present in the ligation reaction in the same molar quantity it is likely that in some positions oligonucleotides from the same parental polynucleotide will ligate next to one another and thus not result in a crossover event. If the concentration of each oligonucleotide from each parent is kept constant during any ligation step in this example, there is a 1/3 chance (assuming 3 parents) that an oligonucleotide from the same parental variant will ligate within the chimeric sequence and produce no crossover.

Accordingly, a probability density function (PDF) can be determined to predict the population of crossover events that are likely to occur during each step in a ligation reaction given a set number of parental variants, a number of oligonucleotides corresponding to each variant, and the concentrations of each variant during each step in the ligation reaction. The statistics and mathematics behind determining the PDF is described below. By utilizing these methods, one can calculate such a probability density function, and thus enrich the chimeric progeny population for a predetermined number of crossover events resulting from a particular ligation reaction. Moreover, a target number of crossover events can be predetermined, and the system then programmed to calculate the starting quantities of each parental oligonucleotide during each step in the ligation reaction to result in a

probability density function that centers on the predetermined number of crossover events. These methods are directed to the use of repeated cycles of reductive reassortment, recombination and selection that allow for the directed molecular evolution of a nucleic acid encoding an polypeptide through recombination. This system allows generation of a large population of evolved chimeric sequences, wherein the generated population is significantly enriched for sequences that have a predetermined number of crossover events. A crossover event is a point in a chimeric sequence where a shift in sequence occurs from one parental variant to another parental variant. Such a point is normally at the juncture of where oligonucleotides from two parents are ligated together to form a single sequence. The method allows calculation of the correct concentrations of oligonucleotide sequences so that the final chimeric population of sequences is enriched for the chosen number of crossover events. This provides more control over choosing chimeric variants having a predetermined number of crossover events.

In addition, these methods provide a convenient means for exploring a tremendous amount of the possible protein variant space in comparison to other systems. By using the methods described herein, the population of chimerics molecules can be enriched for those variants that have a particular number of crossover events. Thus, although one can still generate 10^{13} chimeric molecules during a reaction, each of the molecules chosen for further analysis most likely has, for example, only three crossover events. Because the resulting progeny population can be skewed to have a predetermined number of crossover events, the boundaries on the functional variety between the chimeric molecules is reduced. This provides a more manageable number of variables when calculating which oligonucleotide from the original parental polynucleotides might be responsible for affecting a particular trait.

In one aspect, the method creates a chimeric progeny polynucleotide sequence by creating oligonucleotides corresponding to fragments or portions of each parental sequence. Each oligonucleotide preferably includes a unique region of overlap so that mixing the oligonucleotides together results in a new variant that has each oligonucleotide fragment assembled in the correct order. See also USSN 09/332,835.

The number of oligonucleotides generated for each parental variant bears a relationship to the total number of resulting crossovers in the chimeric molecule that is ultimately created. For example, three parental nucleotide sequence variants might be provided to undergo a ligation reaction in order to find a chimeric variant having, for example, greater activity at high temperature. As one example, a set of 50 oligonucleotide

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Determining Crossover Events

Embodiments of the invention include a system and software that receive a desired crossover probability density function (PDF), the number of parent genes to be reassembled, and the number of fragments in the reassembly as inputs. The output of this program is a "fragment PDF" that can be used to determine a recipe for producing reassembled genes, and the estimated crossover PDF of those genes. The processing described herein is preferably performed in MATLAB® (The Mathworks, Natick, Massachusetts) a programming language and development environment for technical computing.

Iterative Processes

In practicing the invention, these processes can be iteratively repeated. For example a nucleic acid (or, the nucleic acid) responsible for an altered phospholipase

phenotype is identified, re-isolated, again modified, re-tested for activity. This process can be iteratively repeated until a desired phenotype is engineered. For example, an entire biochemical anabolic or catabolic pathway can be engineered into a cell, including phospholipase activity.

Similarly, if it is determined that a particular oligonucleotide has no affect at all on the desired trait (e.g., a new phospholipase phenotype), it can be removed as a variable by synthesizing larger parental oligonucleotides that include the sequence to be removed. Since incorporating the sequence within a larger sequence prevents any crossover events, there will no longer be any variation of this sequence in the progeny polynucleotides. This iterative practice of determining which oligonucleotides are most related to the desired trait, and which are unrelated, allows more efficient exploration all of the possible protein variants that might be provide a particular trait or activity.

In vivo shuffling

In vivo shuffling of molecules is use in methods of the invention that provide variants of polypeptides of the invention, e.g., antibodies, phospholipase enzymes, and the like. *In vivo* shuffling can be performed utilizing the natural property of cells to recombine multimers. While recombination *in vivo* has provided the major natural route to molecular diversity, genetic recombination remains a relatively complex process that involves 1) the recognition of homologies; 2) strand cleavage, strand invasion, and metabolic steps leading to the production of recombinant chiasma; and finally 3) the resolution of chiasma into discrete recombined molecules. The formation of the chiasma requires the recognition of homologous sequences.

In one aspect, the invention provides a method for producing a hybrid polynucleotide from at least a first polynucleotide and a second polynucleotide. The invention can be used to produce a hybrid polynucleotide by introducing at least a first polynucleotide and a second polynucleotide which share at least one region of partial sequence homology into a suitable host cell. The regions of partial sequence homology promote processes which result in sequence reorganization producing a hybrid polynucleotide. The term "hybrid polynucleotide", as used herein, is any nucleotide sequence which results from the method of the present invention and contains sequence from at least two original polynucleotide sequences. Such hybrid polynucleotides can result from intermolecular recombination events which promote sequence integration between DNA molecules. In addition, such hybrid polynucleotides can result from intramolecular reductive

reassortment processes which utilize repeated sequences to alter a nucleotide sequence within a DNA molecule.

Producing sequence variants

The invention also provides methods of making sequence variants of the nucleic acid and phospholipase sequences of the invention or isolating phospholipase enzyme, e.g., phospholipase, sequence variants using the nucleic acids and polypeptides of the invention. In one aspect, the invention provides for variants of an phospholipase gene of the invention, which can be altered by any means, including, e.g., random or stochastic methods, or, non-stochastic, or "directed evolution," methods, as described above.

The isolated variants may be naturally occurring. Variant can also be created *in vitro*. Variants may be created using genetic engineering techniques such as site directed mutagenesis, random chemical mutagenesis, Exonuclease III deletion procedures, and standard cloning techniques. Alternatively, such variants, fragments, analogs, or derivatives may be created using chemical synthesis or modification procedures. Other methods of making variants are also familiar to those skilled in the art. These include procedures in which nucleic acid sequences obtained from natural isolates are modified to generate nucleic acids which encode polypeptides having characteristics which enhance their value in industrial or laboratory applications. In such procedures, a large number of variant sequences having one or more nucleotide differences with respect to the sequence obtained from the natural isolate are generated and characterized. These nucleotide differences can result in amino acid changes with respect to the polypeptides encoded by the nucleic acids from the natural isolates.

For example, variants may be created using error prone PCR. In error prone PCR, PCR is performed under conditions where the copying fidelity of the DNA polymerase is low, such that a high rate of point mutations is obtained along the entire length of the PCR product. Error prone PCR is described, e.g., in Leung, D.W., et al., Technique, 1:11-15, 1989) and Caldwell, R. C. & Joyce G.F., PCR Methods Applic., 2:28-33, 1992. Briefly, in such procedures, nucleic acids to be mutagenized are mixed with PCR primers, reaction buffer, MgCl2, MnCl2, Taq polymerase and an appropriate concentration of dNTPs for achieving a high rate of point mutation along the entire length of the PCR product. For example, the reaction may be performed using 20 fmoles of nucleic acid to be mutagenized, 30pmole of each PCR primer, a reaction buffer comprising 50mM KCl, 10mM Tris HCl (pH 8.3) and 0.01% gelatin, 7mM MgCl2, 0.5mM MnCl2, 5 units of Taq polymerase, 0.2mM

dGTP, 0.2mM dATP, 1mM dCTP, and 1mM dTTP. PCR may be performed for 30 cycles of 94° C for 1 min, 45° C for 1 min, and 72° C for 1 min. However, it will be appreciated that these parameters may be varied as appropriate. The mutagenized nucleic acids are cloned into an appropriate vector and the activities of the polypeptides encoded by the mutagenized nucleic acids is evaluated.

Variants may also be created using oligonucleotide directed mutagenesis to generate site-specific mutations in any cloned DNA of interest. Oligonucleotide mutagenesis is described, e.g., in Reidhaar-Olson (1988) Science 241:53-57. Briefly, in such procedures a plurality of double stranded oligonucleotides bearing one or more mutations to be introduced into the cloned DNA are synthesized and inserted into the cloned DNA to be mutagenized. Clones containing the mutagenized DNA are recovered and the activities of the polypeptides they encode are assessed.

Another method for generating variants is assembly PCR. Assembly PCR involves the assembly of a PCR product from a mixture of small DNA fragments. A large number of different PCR reactions occur in parallel in the same vial, with the products of one reaction priming the products of another reaction. Assembly PCR is described in, e.g., U.S. Patent No. 5,965,408.

Still another method of generating variants is sexual PCR mutagenesis. In sexual PCR mutagenesis, forced homologous recombination occurs between DNA molecules of different but highly related DNA sequence *in vitro*, as a result of random fragmentation of the DNA molecule based on sequence homology, followed by fixation of the crossover by primer extension in a PCR reaction. Sexual PCR mutagenesis is described, e.g., in Stemmer (1994) Proc. Natl. Acad. Sci. USA 91:10747-10751. Briefly, in such procedures a plurality of nucleic acids to be recombined are digested with DNase to generate fragments having an average size of 50-200 nucleotides. Fragments of the desired average size are purified and resuspended in a PCR mixture. PCR is conducted under conditions which facilitate recombination between the nucleic acid fragments. For example, PCR may be performed by resuspending the purified fragments at a concentration of 10-30ng/μl in a solution of 0.2mM of each dNTP, 2.2mM $MgCl_2$, 50mM KCL, 10mM Tris HCl, pH 9.0, and 0.1% Triton X-100. 2.5 units of Taq polymerase per 100:1 of reaction mixture is added and PCR is performed using the following regime: 94°C for 60 seconds, 94°C for 30 seconds, 50-55°C for 30 seconds, 72°C for 30 seconds (30-45 times) and 72°C for 5 minutes. However, it will be appreciated that these parameters may be varied as appropriate. In some aspects, oligonucleotides may be included in the PCR reactions. In other aspects, the Klenow

fragment of DNA polymerase I may be used in a first set of PCR reactions and Taq polymerase may be used in a subsequent set of PCR reactions. Recombinant sequences are isolated and the activities of the polypeptides they encode are assessed.

Variants may also be created by *in vivo* mutagenesis. In some embodiments, random mutations in a sequence of interest are generated by propagating the sequence of interest in a bacterial strain, such as an *E. coli* strain, which carries mutations in one or more of the DNA repair pathways. Such "mutator" strains have a higher random mutation rate than that of a wild-type parent. Propagating the DNA in one of these strains will eventually generate random mutations within the DNA. Mutator strains suitable for use for *in vivo* mutagenesis are described, e.g., in PCT Publication No. WO 91/16427.

Variants may also be generated using cassette mutagenesis. In cassette mutagenesis a small region of a double stranded DNA molecule is replaced with a synthetic oligonucleotide "cassette" that differs from the native sequence. The oligonucleotide often contains completely and/or partially randomized native sequence.

Recursive ensemble mutagenesis may also be used to generate variants. Recursive ensemble mutagenesis is an algorithm for protein engineering (protein mutagenesis) developed to produce diverse populations of phenotypically related mutants whose members differ in amino acid sequence. This method uses a feedback mechanism to control successive rounds of combinatorial cassette mutagenesis. Recursive ensemble mutagenesis is described, e.g., in Arkin (1992) Proc. Natl. Acad. Sci. USA 89:7811-7815.

In some embodiments, variants are created using exponential ensemble mutagenesis. Exponential ensemble mutagenesis is a process for generating combinatorial libraries with a high percentage of unique and functional mutants, wherein small groups of residues are randomized in parallel to identify, at each altered position, amino acids which lead to functional proteins. Exponential ensemble mutagenesis is described, e.g., in Delegrave (1993) Biotechnology Res. 11:1548-1552. Random and site-directed mutagenesis are described, e.g., in Arnold (1993) Current Opinion in Biotechnology 4:450-455.

In some embodiments, the variants are created using shuffling procedures wherein portions of a plurality of nucleic acids which encode distinct polypeptides are fused together to create chimeric nucleic acid sequences which encode chimeric polypeptides as described in, e.g., U.S. Patent Nos. 5,965,408; 5,939,250.

The invention also provides variants of polypeptides of the invention comprising sequences in which one or more of the amino acid residues (e.g., of an exemplary polypeptide of the invention) are substituted with a conserved or non-conserved amino acid

residue (e.g., a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code. Conservative substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Thus, polypeptides of the invention include those with conservative substitutions of sequences of the invention, including but not limited to the following replacements: replacements of an aliphatic amino acid such as Alanine, Valine, Leucine and Isoleucine with another aliphatic amino acid; replacement of a Serine with a Threonine or vice versa; replacement of an acidic residue such as Aspartic acid and Glutamic acid with another acidic residue; replacement of a residue bearing an amide group, such as Asparagine and Glutamine, with another residue bearing an amide group; exchange of a basic residue such as Lysine and Arginine with another basic residue; and replacement of an aromatic residue such as Phenylalanine, Tyrosine with another aromatic residue. Other variants are those in which one or more of the amino acid residues of the polypeptides of the invention includes a substituent group.

Other variants within the scope of the invention are those in which the polypeptide is associated with another compound, such as a compound to increase the half-life of the polypeptide, for example, polyethylene glycol.

Additional variants within the scope of the invention are those in which additional amino acids are fused to the polypeptide, such as a leader sequence, a secretory sequence, a proprotein sequence or a sequence which facilitates purification, enrichment, or stabilization of the polypeptide.

In some aspects, the variants, fragments, derivatives and analogs of the polypeptides of the invention retain the same biological function or activity as the exemplary polypeptides, e.g., a phospholipase activity, as described herein. In other aspects, the variant, fragment, derivative, or analog includes a proprotein, such that the variant, fragment, derivative, or analog can be activated by cleavage of the proprotein portion to produce an active polypeptide.

Optimizing codons to achieve high levels of protein expression in host cells

The invention provides methods for modifying phospholipase-encoding nucleic acids to modify codon usage. In one aspect, the invention provides methods for modifying codons in a nucleic acid encoding a phospholipase to increase or decrease its expression in a host cell. The invention also provides nucleic acids encoding a phospholipase modified to increase its expression in a host cell, phospholipase enzymes so modified, and

methods of making the modified phospholipase enzymes. The method comprises identifying a "non-preferred" or a "less preferred" codon in phospholipase-encoding nucleic acid and replacing one or more of these non-preferred or less preferred codons with a "preferred codon" encoding the same amino acid as the replaced codon and at least one non-preferred or less preferred codon in the nucleic acid has been replaced by a preferred codon encoding the same amino acid. A preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell.

Host cells for expressing the nucleic acids, expression cassettes and vectors of the invention include bacteria, yeast, fungi, plant cells, insect cells and mammalian cells. Thus, the invention provides methods for optimizing codon usage in all of these cells, codon-altered nucleic acids and polypeptides made by the codon-altered nucleic acids. Exemplary host cells include gram negative bacteria, such as *Escherichia coli* and *Pseudomonas fluorescens*; gram positive bacteria, such as *Streptomyces diversa, Lactobacillus gasseri, Lactococcus lactis, Lactococcus cremoris, Bacillus subtilis*. Exemplary host cells also include eukaryotic organisms, e.g., various yeast, such as *Saccharomyces* sp., including *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Pichia pastoris,* and *Kluyveromyces lactis, Hansenula polymorpha, Aspergillus niger*, and mammalian cells and cell lines and insect cells and cell lines. Thus, the invention also includes nucleic acids and polypeptides optimized for expression in these organisms and species.

For example, the codons of a nucleic acid encoding an phospholipase isolated from a bacterial cell are modified such that the nucleic acid is optimally expressed in a bacterial cell different from the bacteria from which the phospholipase was derived, a yeast, a fungi, a plant cell, an insect cell or a mammalian cell. Methods for optimizing codons are well known in the art, see, e.g., U.S. Patent No. 5,795,737; Baca (2000) Int. J. Parasitol. 30:113-118; Hale (1998) Protein Expr. Purif. 12:185-188; Narum (2001) Infect. Immun. 69:7250-7253. See also Narum (2001) Infect. Immun. 69:7250-7253, describing optimizing codons in mouse systems; Outchkourov (2002) Protein Expr. Purif. 24:18-24, describing optimizing codons in yeast; Feng (2000) Biochemistry 39:15399-15409, describing optimizing codons in *E. coli*; Humphreys (2000) Protein Expr. Purif. 20:252-264, describing optimizing codon usage that affects secretion in *E. coli*.

Transgenic non-human animals

The invention provides transgenic non-human animals comprising a nucleic acid, a polypeptide, an expression cassette or vector or a transfected or transformed cell of the invention. The transgenic non-human animals can be, e.g., goats, rabbits, sheep, pigs, cows, rats and mice, comprising the nucleic acids of the invention. These animals can be used, e.g., as *in vivo* models to study phospholipase activity, or, as models to screen for modulators of phospholipase activity *in vivo*. The coding sequences for the polypeptides to be expressed in the transgenic non-human animals can be designed to be constitutive, or, under the control of tissue-specific, developmental-specific or inducible transcriptional regulatory factors. Transgenic non-human animals can be designed and generated using any method known in the art; see, e.g., U.S. Patent Nos. 6,211,428; 6,187,992; 6,156,952; 6,118,044; 6,111,166; 6,107,541; 5,959,171; 5,922,854; 5,892,070; 5,880,327; 5,891,698; 5,639,940; 5,573,933; 5,387,742; 5,087,571, describing making and using transformed cells and eggs and transgenic mice, rats, rabbits, sheep, pigs and cows. See also, e.g., Pollock (1999) J. Immunol. Methods 231:147-157, describing the production of recombinant proteins in the milk of transgenic dairy animals; Baguisi (1999) Nat. Biotechnol. 17:456-461, demonstrating the production of transgenic goats. U.S. Patent No. 6,211,428, describes making and using transgenic non-human mammals which express in their brains a nucleic acid construct comprising a DNA sequence. U.S. Patent No. 5,387,742, describes injecting cloned recombinant or synthetic DNA sequences into fertilized mouse eggs, implanting the injected eggs in pseudo-pregnant females, and growing to term transgenic mice whose cells express proteins related to the pathology of Alzheimer's disease. U.S. Patent No. 6,187,992, describes making and using a transgenic mouse whose genome comprises a disruption of the gene encoding amyloid precursor protein (APP).

"Knockout animals" can also be used to practice the methods of the invention. For example, in one aspect, the transgenic or modified animals of the invention comprise a "knockout animal," e.g., a "knockout mouse," engineered not to express or to be unable to express a phospholipase.

Transgenic Plants and Seeds

The invention provides transgenic plants and seeds comprising a nucleic acid, a polypeptide (e.g., a phospholipase), an expression cassette or vector or a transfected or transformed cell of the invention. The invention also provides plant products, e.g., oils, seeds, leaves, extracts and the like, comprising a nucleic acid and/or a polypeptide (e.g., a phospholipase) of the invention. The transgenic plant can be dicotyledonous (a dicot) or

monocotyledonous (a monocot). The invention also provides methods of making and using these transgenic plants and seeds. The transgenic plant or plant cell expressing a polypeptide of the invention may be constructed in accordance with any method known in the art. See, for example, U.S. Patent No. 6,309,872.

Nucleic acids and expression constructs of the invention can be introduced into a plant cell by any means. For example, nucleic acids or expression constructs can be introduced into the genome of a desired plant host, or, the nucleic acids or expression constructs can be episomes. Introduction into the genome of a desired plant can be such that the host's phospholipase production is regulated by endogenous transcriptional or translational control elements. The invention also provides "knockout plants" where insertion of gene sequence by, e.g., homologous recombination, has disrupted the expression of the endogenous gene. Means to generate "knockout" plants are well-known in the art, see, e.g., Strepp (1998) Proc Natl. Acad. Sci. USA 95:4368-4373; Miao (1995) Plant J 7:359-365. See discussion on transgenic plants, below.

The nucleic acids of the invention can be used to confer desired traits on essentially any plant, e.g., on oil-seed containing plants, such as soybeans, rapeseed, sunflower seeds, sesame and peanuts. Nucleic acids of the invention can be used to manipulate metabolic pathways of a plant in order to optimize or alter host's expression of phospholipase. The can change phospholipase activity in a plant. Alternatively, a phospholipase of the invention can be used in production of a transgenic plant to produce a compound not naturally produced by that plant. This can lower production costs or create a novel product.

In one aspect, the first step in production of a transgenic plant involves making an expression construct for expression in a plant cell. These techniques are well known in the art. They can include selecting and cloning a promoter, a coding sequence for facilitating efficient binding of ribosomes to mRNA and selecting the appropriate gene terminator sequences. One exemplary constitutive promoter is CaMV35S, from the cauliflower mosaic virus, which generally results in a high degree of expression in plants. Other promoters are more specific and respond to cues in the plant's internal or external environment. An exemplary light-inducible promoter is the promoter from the cab gene, encoding the major chlorophyll a/b binding protein.

In one aspect, the nucleic acid is modified to achieve greater expression in a plant cell. For example, a sequence of the invention is likely to have a higher percentage of A-T nucleotide pairs compared to that seen in a plant, some of which prefer G-C nucleotide

pairs. Therefore, A-T nucleotides in the coding sequence can be substituted with G-C nucleotides without significantly changing the amino acid sequence to enhance production of the gene product in plant cells.

Selectable marker gene can be added to the gene construct in order to identify plant cells or tissues that have successfully integrated the transgene. This may be necessary because achieving incorporation and expression of genes in plant cells is a rare event, occurring in just a few percent of the targeted tissues or cells. Selectable marker genes encode proteins that provide resistance to agents that are normally toxic to plants, such as antibiotics or herbicides. Only plant cells that have integrated the selectable marker gene will survive when grown on a medium containing the appropriate antibiotic or herbicide. As for other inserted genes, marker genes also require promoter and termination sequences for proper function.

In one aspect, making transgenic plants or seeds comprises incorporating sequences of the invention and, optionally, marker genes into a target expression construct (e.g., a plasmid), along with positioning of the promoter and the terminator sequences. This can involve transferring the modified gene into the plant through a suitable method. For example, a construct may be introduced directly into the genomic DNA of the plant cell using techniques such as electroporation and microinjection of plant cell protoplasts, or the constructs can be introduced directly to plant tissue using ballistic methods, such as DNA particle bombardment. For example, see, e.g., Christou (1997) Plant Mol. Biol. 35:197-203; Pawlowski (1996) Mol. Biotechnol. 6:17-30; Klein (1987) Nature 327:70-73; Takumi (1997) Genes Genet. Syst. 72:63-69, discussing use of particle bombardment to introduce transgenes into wheat; and Adam (1997) supra, for use of particle bombardment to introduce YACs into plant cells. For example, Rinehart (1997) supra, used particle bombardment to generate transgenic cotton plants. Apparatus for accelerating particles is described U.S. Pat. No. 5,015,580; and, the commercially available BioRad (Biolistics) PDS-2000 particle acceleration instrument; see also, John, U.S. Patent No. 5,608,148; and Ellis, U.S. Patent No. 5, 681,730, describing particle-mediated transformation of gymnosperms.

In one aspect, protoplasts can be immobilized and injected with nucleic acids, e.g., an expression construct. Although plant regeneration from protoplasts is not easy with cereals, plant regeneration is possible in legumes using somatic embryogenesis from protoplast derived callus. Organized tissues can be transformed with naked DNA using gene gun technique, where DNA is coated on tungsten microprojectiles, shot 1/100th the size of cells, which carry the DNA deep into cells and organelles. Transformed tissue is then induced

to regenerate, usually by somatic embryogenesis. This technique has been successful in several cereal species including maize and rice. .

Nucleic acids, e.g., expression constructs, can also be introduced in to plant cells using recombinant viruses. Plant cells can be transformed using viral vectors, such as, e.g., tobacco mosaic virus derived vectors (Rouwendal (1997) Plant Mol. Biol. 33:989-999), see Porta (1996) "Use of viral replicons for the expression of genes in plants," Mol. Biotechnol. 5:209-221.

Alternatively, nucleic acids, e.g., an expression construct, can be combined with suitable T-DNA flanking regions and introduced into a conventional *Agrobacterium tumefaciens* host vector. The virulence functions of the *Agrobacterium tumefaciens* host will direct the insertion of the construct and adjacent marker into the plant cell DNA when the cell is infected by the bacteria. *Agrobacterium tumefaciens*-mediated transformation techniques, including disarming and use of binary vectors, are well described in the scientific literature. See, e.g., Horsch (1984) *Science* 233:496-498; Fraley (1983) *Proc. Natl. Acad. Sci. USA* 80:4803 (1983); *Gene Transfer to Plants*, Potrykus, ed. (Springer-Verlag, Berlin 1995). The DNA in an A. *tumefaciens* cell is contained in the bacterial chromosome as well as in another structure known as a Ti (tumor-inducing) plasmid. The Ti plasmid contains a stretch of DNA termed T-DNA (~20 kb long) that is transferred to the plant cell in the infection process and a series of vir (virulence) genes that direct the infection process. *A. tumefaciens* can only infect a plant through wounds: when a plant root or stem is wounded it gives off certain chemical signals, in response to which, the vir genes of *A. tumefaciens* become activated and direct a series of events necessary for the transfer of the T-DNA from the Ti plasmid to the plant's chromosome. The T-DNA then enters the plant cell through the wound. One speculation is that the T-DNA waits until the plant DNA is being replicated or transcribed, then inserts itself into the exposed plant DNA. In order to use *A. tumefaciens* as a transgene vector, the tumor-inducing section of T-DNA have to be removed, while retaining the T-DNA border regions and the vir genes. The transgene is then inserted between the T-DNA border regions, where it is transferred to the plant cell and becomes integrated into the plant's chromosomes.

The invention provides for the transformation of monocotyledonous plants using the nucleic acids of the invention, including important cereals, see Hiei (1997) Plant Mol. Biol. 35:205-218. See also, *e.g.*, Horsch, Science (1984) 233:496; Fraley (1983) Proc. Natl. Acad. Sci USA 80:4803; Thykjaer (1997) supra; Park (1996) Plant Mol. Biol. 32:1135-1148, discussing T-DNA integration into genomic DNA. See also D'Halluin, U.S.

Patent No. 5,712,135, describing a process for the stable integration of a DNA comprising a gene that is functional in a cell of a cereal, or other monocotyledonous plant.

In one aspect, the third step can involve selection and regeneration of whole plants capable of transmitting the incorporated target gene to the next generation. Such regeneration techniques rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker that has been introduced together with the desired nucleotide sequences. Plant regeneration from cultured protoplasts is described in Evans et al., *Protoplasts Isolation and Culture, Handbook of Plant Cell Culture*, pp. 124-176, MacMillilan Publishing Company, New York, 1983; and Binding, *Regeneration of Plants, Plant Protoplasts*, pp. 21-73, CRC Press, Boca Raton, 1985. Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee (1987) Ann. Rev. of Plant Phys. 38:467-486. To obtain whole plants from transgenic tissues such as immature embryos, they can be grown under controlled environmental conditions in a series of media containing nutrients and hormones, a process known as tissue culture. Once whole plants are generated and produce seed, evaluation of the progeny begins.

After the expression cassette is stably incorporated in transgenic plants, it can be introduced into other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed. Since transgenic expression of the nucleic acids of the invention leads to phenotypic changes, plants comprising the recombinant nucleic acids of the invention can be sexually crossed with a second plant to obtain a final product. Thus, the seed of the invention can be derived from a cross between two transgenic plants of the invention, or a cross between a plant of the invention and another plant. The desired effects (e.g., expression of the polypeptides of the invention to produce a plant in which flowering behavior is altered) can be enhanced when both parental plants express the polypeptides (e.g., a phospholipase) of the invention. The desired effects can be passed to future plant generations by standard propagation means.

The nucleic acids and polypeptides of the invention are expressed in or inserted in any plant or seed. Transgenic plants of the invention can be dicotyledonous or monocotyledonous. Examples of monocot transgenic plants of the invention are grasses, such as meadow grass (blue grass, *Poa*), forage grass such as festuca, lolium, temperate grass, such as *Agrostis*, and cereals, e.g., wheat, oats, rye, barley, rice, sorghum, and maize (corn). Examples of dicot transgenic plants of the invention are tobacco, legumes, such as lupins, potato, sugar beet, pea, bean and soybean, and cruciferous plants (family

Brassicaceae), such as cauliflower, rape seed, and the closely related model organism *Arabidopsis thaliana*. Thus, the transgenic plants and seeds of the invention include a broad range of plants, including, but not limited to, species from the genera *Anacardium, Arachis, Asparagus, Atropa, Avena, Brassica, Citrus, Citrullus, Capsicum, Carthamus, Cocos, Coffea, Cucumis, Cucurbita, Daucus, Elaeis, Fragaria,. Glycine, Gossypium, Helianthus, Heterocallis, Hordeum, Hyoscyamus, Lactuca, Linum, Lolium, Lupinus, Lycopersicon, Malus, Manihot, Majorana, Medicago, Nicotiana, Olea, Oryza, Panieum, Pannisetum, Persea, Phaseolus, Pistachia, Pisum, Pyrus, Prunus, Raphanus, Ricinus, Secale, Senecio, Sinapis, Solanum, Sorghum, Theobromus, Trigonella, Triticum, Vicia, Vitis, Vigna*, and *Zea*.

In alternative embodiments, the nucleic acids of the invention are expressed in plants (e.g., as transgenic plants), such as oil-seed containing plants, e.g., soybeans, rapeseed, sunflower seeds, sesame and peanuts. The nucleic acids of the invention can be expressed in plants which contain fiber cells, including, *e.g.*, cotton, silk cotton tree (Kapok, Ceiba pentandra), desert willow, creosote bush, winterfat, balsa, ramie, kenaf, hemp, roselle, jute, sisal abaca and flax. In alternative embodiments, the transgenic plants of the invention can be members of the genus *Gossypium*, including members of any *Gossypium* species, such as *G. arboreum*;. *G. herbaceum*, *G. barbadense*, and *G. hirsutum*.

The invention also provides for transgenic plants to be used for producing large amounts of the polypeptides (e.g., a phospholipase or antibody) of the invention. For example, see Palmgren (1997) Trends Genet. 13:348; Chong (1997) Transgenic Res. 6:289-296 (producing human milk protein beta-casein in transgenic potato plants using an auxin-inducible, bidirectional mannopine synthase (mas1',2') promoter with *Agrobacterium tumefaciens*-mediated leaf disc transformation methods).

Using known procedures, one of skill can screen for plants of the invention by detecting the increase or decrease of transgene mRNA or protein in transgenic plants. Means for detecting and quantitation of mRNAs or proteins are well known in the art.

Polypeptides and peptides

The invention provides isolated or recombinant polypeptides having a sequence identity (e.g., at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity) to an exemplary sequence of the invention, e.g., SEQ ID NO:2, SEQ ID NO:4, SEQ

ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106. As discussed above, the identity can be over the full length of the polypeptide, or, the identity can be over a subsequence thereof, e.g., a region of at least about 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700 or more residues. Polypeptides of the invention can also be shorter than the full length of exemplary polypeptides (e.g., SEQ ID NO:2; SEQ ID NO:4; SEQ ID NO:6; SEQ ID NO:8, etc.). In alternative embodiment, the invention provides polypeptides (peptides, fragments) ranging in size between about 5 and the full length of a polypeptide, e.g., an enzyme, such as a phospholipase, e.g., phospholipase; exemplary sizes being of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 125, 150, 175, 200, 250, 300, 350, 400 or more residues, e.g., contiguous residues of the exemplary phospholipases of SEQ ID NO:2; SEQ ID NO:4; SEQ ID NO:6; SEQ ID NO:8, etc.. Peptides of the invention can be useful as, e.g., labeling probes, antigens, toleragens, motifs, phospholipase active sites.

In one aspect, the polypeptide has a phospholipase activity, e.g., cleavage of a glycerolphosphate ester linkage, the ability to hydrolyze phosphate ester bonds, including patatin, lipid acyl hydrolase (LAH), phospholipase A, B, C and/or phospholipase D activity. In one aspect, exemplary polypeptides of the invention have a phospholipase activity as set forth in Table 1, below:

Table 1

SEQ ID NO:	Enzyme type
103, 104	Patatin
11, 12	Patatin
13, 14	Patatin
17, 18	Patatin
25, 26	Patatin
27, 28	Patatin
33, 34	Patatin

35, 36	Patatin
43, 44	Patatin
45, 46	Patatin
55, 56	Patatin
59, 60	Patatin
65, 66	Patatin
71, 72	Patatin
77, 78	Patatin
86, 87	Patatin
87, 88	Patatin
91, 92	Patatin
95, 96	Patatin
99, 100	Patatin
1, 2	PLC
101, 102	PLC
105, 106	PLC
3, 4	PLC
31, 32	PLC
5, 6	PLC
7, 8	PLC
81, 82	PLC
89, 90	PLC
9, 10	PLC
93, 94	PLC
97, 98	PLC
15, 16	PLD
19, 20	PLD
21, 22	PLD
23, 24	PLD
29, 30	PLD
37, 38	PLD
39, 40	PLD
41, 42	PLD
47, 48	PLD
49, 50	PLD
51, 52	PLD
53, 54	PLD
57, 58	PLD
61, 62	PLD
63, 64	PLD

67, 68	PLD
71, 72	PLD
73, 74	PLD
75, 76	PLD
79, 80	PLD
83, 84	PLD

Polypeptides and peptides of the invention can be isolated from natural sources, be synthetic, or be recombinantly generated polypeptides. Peptides and proteins can be recombinantly expressed *in vitro* or *in vivo*. The peptides and polypeptides of the invention can be made and isolated using any method known in the art. Polypeptide and peptides of the invention can also be synthesized, whole or in part, using chemical methods well known in the art. See e.g., Caruthers (1980) Nucleic Acids Res. Symp. Ser. 215-223; Horn (1980) Nucleic Acids Res. Symp. Ser. 225-232; Banga, A.K., Therapeutic Peptides and Proteins, Formulation, Processing and Delivery Systems (1995) Technomic Publishing Co., Lancaster, PA. For example, peptide synthesis can be performed using various solid-phase techniques (see e.g., Roberge (1995) Science 269:202; Merrifield (1997) Methods Enzymol. 289:3□13) and automated synthesis may be achieved, e.g., using the ABI 431A Peptide Synthesizer (Perkin Elmer) in accordance with the instructions provided by the manufacturer.

The peptides and polypeptides of the invention can also be glycosylated. The glycosylation can be added post-translationally either chemically or by cellular biosynthetic mechanisms, wherein the later incorporates the use of known glycosylation motifs, which can be native to the sequence or can be added as a peptide or added in the nucleic acid coding sequence. The glycosylation can be O-linked or N-linked.

The peptides and polypeptides of the invention, as defined above, include all "mimetic" and "peptidomimetic" forms. The terms "mimetic" and "peptidomimetic" refer to a synthetic chemical compound which has substantially the same structural and/or functional characteristics of the polypeptides of the invention. The mimetic can be either entirely composed of synthetic, non-natural analogues of amino acids, or, is a chimeric molecule of partly natural peptide amino acids and partly non-natural analogs of amino acids. The mimetic can also incorporate any amount of natural amino acid conservative substitutions as long as such substitutions also do not substantially alter the mimetic's structure and/or activity. As with polypeptides of the invention which are conservative variants, routine experimentation will determine whether a mimetic is within the scope of the invention, i.e.,

that its structure and/or function is not substantially altered. Thus, in one aspect, a mimetic composition is within the scope of the invention if it has a phospholipase activity.

Polypeptide mimetic compositions of the invention can contain any combination of non-natural structural components. In alternative aspect, mimetic compositions of the invention include one or all of the following three structural groups: a) residue linkage groups other than the natural amide bond ("peptide bond") linkages; b) non-natural residues in place of naturally occurring amino acid residues; or c) residues which induce secondary structural mimicry, i.e., to induce or stabilize a secondary structure, e.g., a beta turn, gamma turn, beta sheet, alpha helix conformation, and the like. For example, a polypeptide of the invention can be characterized as a mimetic when all or some of its residues are joined by chemical means other than natural peptide bonds. Individual peptidomimetic residues can be joined by peptide bonds, other chemical bonds or coupling means, such as, e.g., glutaraldehyde, N-hydroxysuccinimide esters, bifunctional maleimides, N,N'-dicyclohexylcarbodiimide (DCC) or N,N'-diisopropylcarbodiimide (DIC). Linking groups that can be an alternative to the traditional amide bond ("peptide bond") linkages include, e.g., ketomethylene (e.g., -C(=O)-CH2- for -C(=O)-NH-), aminomethylene (CH2-NH), ethylene, olefin (CH=CH), ether (CH2-O), thioether (CH2-S), tetrazole (CN4-), thiazole, retroamide, thioamide, or ester (see, e.g., Spatola (1983) in Chemistry and Biochemistry of Amino Acids, Peptides and Proteins, Vol. 7, pp 267-357, "Peptide Backbone Modifications," Marcell Dekker, NY).

A polypeptide of the invention can also be characterized as a mimetic by containing all or some non-natural residues in place of naturally occurring amino acid residues. Non-natural residues are well described in the scientific and patent literature; a few exemplary non-natural compositions useful as mimetics of natural amino acid residues and guidelines are described below. Mimetics of aromatic amino acids can be generated by replacing by, e.g., D- or L- naphylalanine; D- or L- phenylglycine; D- or L-2 thieneylalanine; D- or L-1, -2, 3-, or 4- pyreneylalanine; D- or L-3 thieneylalanine; D- or L-(2-pyridinyl)-alanine; D- or L-(3-pyridinyl)-alanine; D- or L-(2-pyrazinyl)-alanine; D- or L-(4-isopropyl)-phenylglycine; D-(trifluoromethyl)-phenylglycine; D-(trifluoromethyl)-phenylalanine; D-p-fluoro-phenylalanine; D- or L-p-biphenylphenylalanine; K- or L-p-methoxy-biphenylphenylalanine; D- or L-2-indole(alkyl)alanines; and, D- or L-alkylainines, where alkyl can be substituted or unsubstituted methyl, ethyl, propyl, hexyl, butyl, pentyl, isopropyl, iso-butyl, sec-isotyl, iso-pentyl, or a non-acidic amino acids. Aromatic rings of a non-natural

amino acid include, e.g., thiazolyl, thiophenyl, pyrazolyl, benzimidazolyl, naphthyl, furanyl, pyrrolyl, and pyridyl aromatic rings.

Mimetics of acidic amino acids can be generated by substitution by, e.g., non-carboxylate amino acids while maintaining a negative charge; (phosphono)alanine; sulfated threonine. Carboxyl side groups (e.g., aspartyl or glutamyl) can also be selectively modified by reaction with carbodiimides (R'-N-C-N-R') such as, e.g., 1-cyclohexyl-3(2-morpholinyl-(4-ethyl) carbodiimide or 1-ethyl-3(4-azonia- 4,4- dimetholpentyl) carbodiimide. Aspartyl or glutamyl can also be converted to asparaginyl and glutaminyl residues by reaction with ammonium ions. Mimetics of basic amino acids can be generated by substitution with, e.g., (in addition to lysine and arginine) the amino acids ornithine, citrulline, or (guanidino)-acetic acid, or (guanidino)alkyl-acetic acid, where alkyl is defined above. Nitrile derivative (e.g., containing the CN-moiety in place of COOH) can be substituted for asparagine or glutamine. Asparaginyl and glutaminyl residues can be deaminated to the corresponding aspartyl or glutamyl residues. Arginine residue mimetics can be generated by reacting arginyl with, e.g., one or more conventional reagents, including, e.g., phenylglyoxal, 2,3-butanedione, 1,2-cyclo-hexanedione, or ninhydrin, preferably under alkaline conditions. Tyrosine residue mimetics can be generated by reacting tyrosyl with, e.g., aromatic diazonium compounds or tetranitromethane. N-acetylimidizol and tetranitromethane can be used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Cysteine residue mimetics can be generated by reacting cysteinyl residues with, e.g., alpha-haloacetates such as 2-chloroacetic acid or chloroacetamide and corresponding amines; to give carboxymethyl or carboxyamidomethyl derivatives. Cysteine residue mimetics can also be generated by reacting cysteinyl residues with, e.g., bromo-trifluoroacetone, alpha-bromo-beta-(5-imidozoyl) propionic acid; chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide; methyl 2-pyridyl disulfide; p-chloromercuribenzoate; 2-chloromercuri-4 nitrophenol; or, chloro-7-nitrobenzo-oxa-1,3-diazole. Lysine mimetics can be generated (and amino terminal residues can be altered) by reacting lysinyl with, e.g., succinic or other carboxylic acid anhydrides. Lysine and other alpha-amino-containing residue mimetics can also be generated by reaction with imidoesters, such as methyl picolinimidate, pyridoxal phosphate, pyridoxal, chloroborohydride, trinitro-benzenesulfonic acid, O-methylisourea, 2,4, pentanedione, and transamidase-catalyzed reactions with glyoxylate. Mimetics of methionine can be generated by reaction with, e.g., methionine sulfoxide. Mimetics of proline include, e.g., pipecolic acid, thiazolidine carboxylic acid, 3- or 4- hydroxy proline, dehydroproline, 3- or 4-methylproline, or 3,3,-dimethylproline. Histidine residue mimetics can be generated by

reacting histidyl with, e.g., diethylprocarbonate or para-bromophenacyl bromide. Other mimetics include, e.g., those generated by hydroxylation of proline and lysine; phosphorylation of the hydroxyl groups of seryl or threonyl residues; methylation of the alpha-amino groups of lysine, arginine and histidine; acetylation of the N-terminal amine; methylation of main chain amide residues or substitution with N-methyl amino acids; or amidation of C-terminal carboxyl groups.

A residue, e.g., an amino acid, of a polypeptide of the invention can also be replaced by an amino acid (or peptidomimetic residue) of the opposite chirality. Thus, any amino acid naturally occurring in the L-configuration (which can also be referred to as the R or S, depending upon the structure of the chemical entity) can be replaced with the amino acid of the same chemical structural type or a peptidomimetic, but of the opposite chirality, referred to as the D- amino acid, but also can be referred to as the R- or S- form.

The invention also provides methods for modifying the polypeptides of the invention by either natural processes, such as post-translational processing (e.g., phosphorylation, acylation, etc), or by chemical modification techniques, and the resulting modified polypeptides. Modifications can occur anywhere in the polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also a given polypeptide may have many types of modifications. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of a phosphatidylinositol, cross-linking cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristolyation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, and transfer-RNA mediated addition of amino acids to protein such as arginylation. See, e.g., Creighton, T.E., Proteins – Structure and Molecular Properties 2nd Ed., W.H. Freeman and Company, New York (1993); Posttranslational Covalent Modification of Proteins, B.C. Johnson, Ed., Academic Press, New York, pp. 1-12 (1983).

Solid-phase chemical peptide synthesis methods can also be used to synthesize the polypeptide or fragments of the invention. Such method have been known in the art since the early 1960's (Merrifield, R. B., J. Am. Chem. Soc., 85:2149-2154, 1963) (See also

Stewart, J. M. and Young, J. D., Solid Phase Peptide Synthesis, 2nd Ed., Pierce Chemical Co., Rockford, Ill., pp. 11-12)) and have recently been employed in commercially available laboratory peptide design and synthesis kits (Cambridge Research Biochemicals). Such commercially available laboratory kits have generally utilized the teachings of H. M. Geysen et al, Proc. Natl. Acad. Sci., USA, 81:3998 (1984) and provide for synthesizing peptides upon the tips of a multitude of "rods" or "pins" all of which are connected to a single plate. When such a system is utilized, a plate of rods or pins is inverted and inserted into a second plate of corresponding wells or reservoirs, which contain solutions for attaching or anchoring an appropriate amino acid to the pin's or rod's tips. By repeating such a process step, i.e., inverting and inserting the rod's and pin's tips into appropriate solutions, amino acids are built into desired peptides. In addition, a number of available FMOC peptide synthesis systems are available. For example, assembly of a polypeptide or fragment can be carried out on a solid support using an Applied Biosystems, Inc. Model 431A™ automated peptide synthesizer. Such equipment provides ready access to the peptides of the invention, either by direct synthesis or by synthesis of a series of fragments that can be coupled using other known techniques.

Phospholipase enzymes

The invention provides novel phospholipases, nucleic acids encoding them, antibodies that bind them, peptides representing the enzyme's antigenic sites (epitopes) and active sites, and methods for making and using them. In one aspect, polypeptides of the invention have a phospholipase activity, as described above (e.g., cleavage of a glycerolphosphate ester linkage). In alternative aspects, the phospholipases of the invention have activities that have been modified from those of the exemplary phospholipases described herein. The invention includes phospholipases with and without signal sequences and the signal sequences themselves. The invention includes immobilized phospholipases, anti-phospholipase antibodies and fragments thereof. The invention includes heterocomplexes, e.g., fusion proteins, heterodimers, etc., comprising the phospholipases of the invention.

Determining peptides representing the enzyme's antigenic sites (epitopes), active sites, binding sites, signal sequences, and the like can be done by routine screening protocols.

The enzymes of the invention are highly selective catalysts. As with other enzymes, they catalyze reactions with exquisite stereo-, regio-, and chemo- selectivities that

are unparalleled in conventional synthetic chemistry. Moreover, the enzymes of the invention are remarkably versatile. They can be tailored to function in organic solvents, operate at extreme pHs (for example, high pHs and low pHs) extreme temperatures (for example, high temperatures and low temperatures), extreme salinity levels (for example, high salinity and low salinity), and catalyze reactions with compounds that are structurally unrelated to their natural, physiological substrates. Enzymes of the invention can be designed to be reactive toward a wide range of natural and unnatural substrates, thus enabling the modification of virtually any organic lead compound. Enzymes of the invention can also be designed to be highly enantio- and regio-selective. The high degree of functional group specificity exhibited by these enzymes enables one to keep track of each reaction in a synthetic sequence leading to a new active compound. Enzymes of the invention can also be designed to catalyze many diverse reactions unrelated to their native physiological function in nature.

The present invention exploits the unique catalytic properties of enzymes. Whereas the use of biocatalysts (i.e., purified or crude enzymes, non-living or living cells) in chemical transformations normally requires the identification of a particular biocatalyst that reacts with a specific starting compound. The present invention uses selected biocatalysts, i.e., the enzymes of the invention, and reaction conditions that are specific for functional groups that are present in many starting compounds. Each biocatalyst is specific for one functional group, or several related functional groups, and can react with many starting compounds containing this functional group. The biocatalytic reactions produce a population of derivatives from a single starting compound. These derivatives can be subjected to another round of biocatalytic reactions to produce a second population of derivative compounds. Thousands of variations of the original compound can be produced with each iteration of biocatalytic derivatization.

Enzymes react at specific sites of a starting compound without affecting the rest of the molecule, a process that is very difficult to achieve using traditional chemical methods. This high degree of biocatalytic specificity provides the means to identify a single active enzyme within a library. The library is characterized by the series of biocatalytic reactions used to produce it, a so-called "biosynthetic history". Screening the library for biological activities and tracing the biosynthetic history identifies the specific reaction sequence producing the active compound. The reaction sequence is repeated and the structure of the synthesized compound determined. This mode of identification, unlike other synthesis and screening approaches, does not require immobilization technologies, and

compounds can be synthesized and tested free in solution using virtually any type of screening assay. It is important to note, that the high degree of specificity of enzyme reactions on functional groups allows for the "tracking" of specific enzymatic reactions that make up the biocatalytically produced library.

The invention also provides methods of discovering new phospholipases using the nucleic acids, polypeptides and antibodies of the invention. In one aspect, lambda phage libraries are screened for expression-based discovery of phospholipases. Use of lambda phage libraries in screening allows detection of toxic clones; improved access to substrate; reduced need for engineering a host, by-passing the potential for any bias resulting from mass excision of the library; and, faster growth at low clone densities. Screening of lambda phage libraries can be in liquid phase or in solid phase. Screening in liquid phase gives greater flexibility in assay conditions; additional substrate flexibility; higher sensitivity for weak clones; and ease of automation over solid phase screening.

Many of the procedural steps are performed using robotic automation enabling the execution of many thousands of biocatalytic reactions and screening assays per day as well as ensuring a high level of accuracy and reproducibility (see discussion of arrays, below). As a result, a library of derivative compounds can be produced in a matter of weeks. For further teachings on modification of molecules, including small molecules, see PCT/US94/09174.

Phospholipase signal sequences and catalytic domains

The invention provides phospholipase signal sequences (e.g., signal peptides (SPs)) and catalytic domains (CDs). The invention provides nucleic acids encoding these catalytic domains (CDs) and signal sequences (SPs, e.g., a peptide having a sequence comprising/ consisting of amino terminal residues of a polypeptide of the invention). In one aspect, the invention provides a signal sequence comprising a peptide comprising/ consisting of a sequence as set forth in residues 1 to 20, 1 to 21, 1 to 22, 1 to 23, 1 to 24, 1 to 25, 1 to 26, 1 to 27, 1 to 28, 1 to 28, 1 to 30, 1 to 31, 1 to 32 or 1 to 33 of a polypeptide of the invention, e.g., SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66,

SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106.

Exemplary signal sequences are set forth in the SEQ ID listing, e.g., residues 1 to 24 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6; residues 1 to 29 of SEQ ID NO:8; residues 1 to 20 of SEQ ID NO:10; residues 1 to 19 of SEQ ID NO:20; residues 1 to 28 of SEQ ID NO:22; residues 1 to 20 of SEQ ID NO:32; residues 1 to 23 of SEQ ID NO:38; see SEQ ID listing for other exemplary signal sequences of the invention.

The phospholipase signal sequences of the invention can be isolated peptides, or, sequences joined to another phospholipase or a non-phospholipase polypeptide, e.g., as a fusion protein. In one aspect, the invention provides polypeptides comprising phospholipase signal sequences of the invention. In one aspect, polypeptides comprising phospholipase signal sequences of the invention comprise sequences heterologous to a phospholipase of the invention (e.g., a fusion protein comprising a phospholipase signal sequence of the invention and sequences from another phospholipase or a non-phospholipase protein). In one aspect, the invention provides phospholipases of the invention with heterologous signal sequences, e.g., sequences with a yeast signal sequence. A phospholipase of the invention can comprise a heterologous signal sequence, e.g., in a vector, e.g., a pPIC series vector (Invitrogen, Carlsbad, CA).

In one aspect, the signal sequences of the invention are identified following identification of novel phospholipase polypeptides. The pathways by which proteins are sorted and transported to their proper cellular location are often referred to as protein targeting pathways. One of the most important elements in all of these targeting systems is a short amino acid sequence at the amino terminus of a newly synthesized polypeptide called the signal sequence. This signal sequence directs a protein to its appropriate location in the cell and is removed during transport or when the protein reaches its final destination. Most lysosomal, membrane, or secreted proteins have an amino-terminal signal sequence that marks them for translocation into the lumen of the endoplasmic reticulum. More than 100 signal sequences for proteins in this group have been determined. The signal sequences can vary in length from 13 to 36 amino acid residues. Various methods of recognition of signal sequences are known to those of skill in the art. For example, in one aspect, novel phospholipase signal peptides are identified by a method referred to as SignalP. SignalP uses a combined neural network which recognizes both signal peptides and their cleavage sites.

(Nielsen, et al., "Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites." Protein Engineering, vol. 10, no. 1, p. 1-6 (1997).

It should be understood that in some aspects phospholipases of the invention may not have signal sequences. In one aspect, the invention provides the phospholipases of the invention lacking all or part of a signal sequence. In one aspect, the invention provides a nucleic acid sequence encoding a signal sequence from one phospholipase operably linked to a nucleic acid sequence of a different phospholipase or, optionally, a signal sequence from a non-phospholipase protein may be desired.

The invention also provides isolated or recombinant polypeptides comprising signal sequences (SPs) and catalytic domains (CDs) of the invention and heterologous sequences. The heterologous sequences are sequences not naturally associated (e.g., to a phospholipase) with an SP and/or CD. The sequence to which the SP and/or CD are not naturally associated can be on the SP's, and/or CD's amino terminal end, carboxy terminal end, and/or on both ends of the SP and/or CD. In one aspect, the invention provides an isolated or recombinant polypeptide comprising (or consisting of) a polypeptide comprising a signal sequence (SP) and/or catalytic domain (CD) of the invention with the proviso that it is not associated with any sequence to which it is naturally associated (e.g., a phospholipase sequence). Similarly in one aspect, the invention provides isolated or recombinant nucleic acids encoding these polypeptides. Thus, in one aspect, the isolated or recombinant nucleic acid of the invention comprises coding sequence for a signal sequence (SP) and/or catalytic domain (CD) of the invention and a heterologous sequence (i.e., a sequence not naturally associated with the a signal sequence (SP) and/or catalytic domain (CD) of the invention). The heterologous sequence can be on the 3' terminal end, 5' terminal end, and/or on both ends of the SP and/or CD coding sequence.

Assays for phospholipase activity

The invention provides isolated or recombinant polypeptides having a phospholipase activity and nucleic acids encoding them. Any of the many phospholipase activity assays known in the art can be used to determinine if a polypeptide has a phospholipase activity and is within the scope of the invention. Routine protocols for determining phospholipase A, B, D and C, patatin and lipid acyl hydrolase activities are well known in the art.

Exemplary activity assays include turbidity assays, methylumbelliferyl phosphocholine (fluorescent) assays, Amplex red (fluorescent) phospholipase assays, thin

layer chromatography assays (TLC), cytolytic assays and p-nitrophenylphosphorylcholine assays. Using these assays polypeptides can be quickly screened for phospholipase activity.

The phospholipase activity can comprise a lipid acyl hydrolase (LAH) activity. See, e.g., Jimenez (2001) Lipids 36:1169-1174, describing an octaethylene glycol monododecyl ether-based mixed micellar assay for determining the lipid acyl hydrolase activity of a patatin. Pinsirodom (2000) J. Agric. Food Chem. 48:155-160, describes an exemplary lipid acyl hydrolase (LAH) patatin activity.

Turbidity assays to determine phospholipase activity are described, e.g., in Kauffmann (2001) "Conversion of *Bacillus thermocatenulatus* lipase into an efficient phospholipase with increased activity towards long-chain fatty acyl substrates by directed evolution and rational design," Protein Engineering 14:919-928; Ibrahim (1995) "Evidence implicating phospholipase as a virulence factor of *Candida albicans*," Infect. Immun. 63:1993-1998.

Methylumbelliferyl (fluorescent) phosphocholine assays to determine phospholipase activity are described, e.g., in Goode (1997) "Evidence for cell surface and internal phospholipase activity in ascidian eggs," Develop. Growth Differ. 39:655-660; Diaz (1999) "Direct fluorescence-based lipase activity assay," BioTechniques 27:696-700.

Amplex Red (fluorescent) Phospholipase Assays to determine phospholipase activity are available as kits, e.g., the detection of phosphatidylcholine-specific phospholipase using an Amplex Red phosphatidylcholine-specific phospholipase assay kit from Molecular Probes Inc. (Eugene, OR), according to manufacturer's instructions. Fluorescence is measured in a fluorescence microplate reader using excitation at 560 ± 10 nm and fluorescence detection at 590 ± 10 nm. The assay is sensitive at very low enzyme concentrations.

Thin layer chromatography assays (TLC) to determine phospholipase activity are described, e.g., in Reynolds (1991) Methods in Enzymol. 197:3-13; Taguchi (1975) "Phospholipase from *Clostridium novyi* type A.I," Biochim. Biophys. Acta 409:75-85. Thin layer chromatography (TLC) is a widely used technique for detection of phospholipase activity. Various modifications of this method have been used to extract the phospholipids from the aqueous assay mixtures. In some PLC assays the hydrolysis is stopped by addition of chloroform/methanol (2:1) to the reaction mixture. The unreacted starting material and the diacylglycerol are extracted into the organic phase and may be fractionated by TLC, while the head group product remains in the aqueous phase. For more precise measurement of the phospholipid digestion, radiolabeled substrates can be used (see, e.g., Reynolds (1991)

Methods in Enzymol. 197:3-13). The ratios of products and reactants can be used to calculate the actual number of moles of substrate hydrolyzed per unit time. If all the components are extracted equally, any losses in the extraction will affect all components equally. Separation of phospholipid digestion products can be achieved by silica gel TLC with chloroform/methanol/water (65:25:4) used as a solvent system (see, e.g., Taguchi (1975) Biochim. Biophys. Acta 409:75-85).

p-Nitrophenylphosphorylcholine assays to determine phospholipase activity are described, e.g., in Korbsrisate (1999) "Cloning and characterization of a nonhemolytic phospholipase gene from *Burkholderia pseudomallei*," J. Clin. Microbiol. 37:3742-3745; Berka (1981) "Studies of phospholipase (heat labile hemolysin) in *Pseudomonas aeroginosa*," Infect. Immun. 34:1071-1074. This assay is based on enzymatic hydrolysis of the substrate analog p-nitrophenylphosphorylcholine to liberate a yellow chromogenic compound p-nitrophenol, detectable at 405 nm. This substrate is convenient for high-throughput screening.

A cytolytic assay can detect phospholipases with cytolytic activity based on lysis of erythrocytes. Toxic phospholipases can interact with eukaryotic cell membranes and hydrolyze phosphatidylcholine and sphingomyelin, leading to cell lysis. See, e.g., Titball (1993) Microbiol. Rev. 57:347-366.

Hybrid (chimeric) phospholipases and peptide libraries

In one aspect, the invention provides hybrid phospholipases and fusion proteins, including peptide libraries, comprising sequences of the invention. The peptide libraries of the invention can be used to isolate peptide modulators (e.g., activators or inhibitors) of targets, such as phospholipase substrates, receptors, enzymes. The peptide libraries of the invention can be used to identify formal binding partners of targets, such as ligands, e.g., cytokines, hormones and the like. In one aspect, the invention provides chimeric proteins comprising a signal sequence (SP) and/or catalytic domain (CD) of the invention and a heterologous sequence (see above).

The invention also provides methods for generating "improved" and hybrid phospholipases using the nucleic acids and polypeptides of the invention. For example, the invention provides methods for generating enzymes that have activity, e.g., phospholipase activity (such as, e.g., phospholipase A, B, C or D activity, patatin esterase activity, cleavage of a glycerolphosphate ester linkage, cleavage of an ester linkage in a phospholipid in a vegetable oil) at extreme alkaline pHs and/or acidic pHs, high and low temperatures, osmotic

conditions and the like. The invention provides methods for generating hybrid enzymes (e.g., hybrid phospholipases).

In one aspect, the methods of the invention produce new hybrid polypeptides by utilizing cellular processes that integrate the sequence of a first polynucleotide such that resulting hybrid polynucleotides encode polypeptides demonstrating activities derived from the first biologically active polypeptides. For example, the first polynucleotides can be an exemplary nucleic acid sequence (e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, etc.) encoding an exemplary phospholipase of the invention (e.g., SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, etc.). The first nucleic acid can encode an enzyme from one organism that functions effectively under a particular environmental condition, e.g. high salinity. It can be "integrated" with an enzyme encoded by a second polynucleotide from a different organism that functions effectively under a different environmental condition, such as extremely high temperatures. For example, when the two nucleic acids can produce a hybrid molecule by e.g., recombination and/or reductive reassortment. A hybrid polynucleotide containing sequences from the first and second original polynucleotides may encode an enzyme that exhibits characteristics of both enzymes encoded by the original polynucleotides. Thus, the enzyme encoded by the hybrid polynucleotide may function effectively under environmental conditions shared by each of the enzymes encoded by the first and second polynucleotides, e.g., high salinity and extreme temperatures.

Alternatively, a hybrid polypeptide resulting from this method of the invention may exhibit specialized enzyme activity not displayed in the original enzymes. For example, following recombination and/or reductive reassortment of polynucleotides encoding phospholipase activities, the resulting hybrid polypeptide encoded by a hybrid polynucleotide can be screened for specialized activities obtained from each of the original enzymes, i.e. the type of bond on which the phospholipase acts and the temperature at which the phospholipase functions. Thus, for example, the phospholipase may be screened to ascertain those chemical functionalities which distinguish the hybrid phospholipase from the original phospholipases, such as: (a) amide (peptide bonds), i.e., phospholipases; (b) ester bonds, i.e., amylases and lipases; (c) acetals, i.e., glycosidases and, for example, the temperature, pH or salt concentration at which the hybrid polypeptide functions.

Sources of the polynucleotides to be "integrated" with nucleic acids of the invention may be isolated from individual organisms ("isolates"), collections of organisms that have been grown in defined media ("enrichment cultures"), or, uncultivated organisms ("environmental samples"). The use of a culture-independent approach to derive

polynucleotides encoding novel bioactivities from environmental samples is most preferable since it allows one to access untapped resources of biodiversity. "Environmental libraries" are generated from environmental samples and represent the collective genomes of naturally occurring organisms archived in cloning vectors that can be propagated in suitable prokaryotic hosts. Because the cloned DNA is initially extracted directly from environmental samples, the libraries are not limited to the small fraction of prokaryotes that can be grown in pure culture. Additionally, a normalization of the environmental DNA present in these samples could allow more equal representation of the DNA from all of the species present in the original sample. This can dramatically increase the efficiency of finding interesting genes from minor constituents of the sample that may be under-represented by several orders of magnitude compared to the dominant species.

For example, gene libraries generated from one or more uncultivated microorganisms are screened for an activity of interest. Potential pathways encoding bioactive molecules of interest are first captured in prokaryotic cells in the form of gene expression libraries. Polynucleotides encoding activities of interest are isolated from such libraries and introduced into a host cell. The host cell is grown under conditions that promote recombination and/or reductive reassortment creating potentially active biomolecules with novel or enhanced activities.

The microorganisms from which hybrid polynucleotides may be prepared include prokaryotic microorganisms, such as *Eubacteria* and *Archaebacteria,* and lower eukaryotic microorganisms such as fungi, some algae and protozoa. Polynucleotides may be isolated from environmental samples. Nucleic acid may be recovered without culturing of an organism or recovered from one or more cultured organisms. In one aspect, such microorganisms may be extremophiles, such as hyperthermophiles, psychrophiles, psychrotrophs, halophiles, barophiles and acidophiles. In one aspect, polynucleotides encoding phospholipase enzymes isolated from extremophilic microorganisms are used to make hybrid enzymes. Such enzymes may function at temperatures above 100°C in, e.g., terrestrial hot springs and deep sea thermal vents, at temperatures below 0°C in, e.g., arctic waters, in the saturated salt environment of, e.g., the Dead Sea, at pH values around 0 in, e.g., coal deposits and geothermal sulfur-rich springs, or at pH values greater than 11 in, e.g., sewage sludge. For example, phospholipases cloned and expressed from extremophilic organisms can show high activity throughout a wide range of temperatures and pHs.

Polynucleotides selected and isolated as described herein, including at least one nucleic acid of the invention, are introduced into a suitable host cell. A suitable host cell is any cell that is capable of promoting recombination and/or reductive reassortment. The selected polynucleotides can be in a vector that includes appropriate control sequences. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or preferably, the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis et al., 1986).

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as *E. coli*, *Streptomyces*, *Salmonella typhimurium*; fungal cells, such as yeast; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9; animal cells such as CHO, COS or *Bowes melanoma*; adenoviruses; and plant cells. The selection of an appropriate host for recombination and/or reductive reassortment or just for expression of recombinant protein is deemed to be within the scope of those skilled in the art from the teachings herein. Mammalian cell culture systems that can be employed for recombination and/or reductive reassortment or just for expression of recombinant protein include, e.g., the COS-7 lines of monkey kidney fibroblasts, described in "SV40-transformed simian cells support the replication of early SV40 mutants" (Gluzman, 1981), the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors can comprise an origin of replication, a suitable promoter and enhancer, and necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required non-transcribed genetic elements.

Host cells containing the polynucleotides of interest (for recombination and/or reductive reassortment or just for expression of recombinant protein) can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan. The clones which are identified as having the specified enzyme activity may then be sequenced to identify the polynucleotide sequence encoding an enzyme having the enhanced activity.

In another aspect, the nucleic acids and methods of the present invention can be used to generate novel polynucleotides for biochemical pathways, e.g., pathways from one or more operons or gene clusters or portions thereof. For example, bacteria and many

eukaryotes have a coordinated mechanism for regulating genes whose products are involved in related processes. The genes are clustered, in structures referred to as "gene clusters," on a single chromosome and are transcribed together under the control of a single regulatory sequence, including a single promoter which initiates transcription of the entire cluster. Thus, a gene cluster is a group of adjacent genes that are either identical or related, usually as to their function.

Gene cluster DNA can be isolated from different organisms and ligated into vectors, particularly vectors containing expression regulatory sequences which can control and regulate the production of a detectable protein or protein-related array activity from the ligated gene clusters. Use of vectors which have an exceptionally large capacity for exogenous DNA introduction are particularly appropriate for use with such gene clusters and are described by way of example herein to include the f-factor (or fertility factor) of *E. coli.* This f-factor of *E. coli* is a plasmid which affects high-frequency transfer of itself during conjugation and is ideal to achieve and stably propagate large DNA fragments, such as gene clusters from mixed microbial samples. "Fosmids," cosmids or bacterial artificial chromosome (BAC) vectors can be used as cloning vectors. These are derived from *E. coli* f-factor which is able to stably integrate large segments of genomic DNA. When integrated with DNA from a mixed uncultured environmental sample, this makes it possible to achieve large genomic fragments in the form of a stable "environmental DNA library." Cosmid vectors were originally designed to clone and propagate large segments of genomic DNA. Cloning into cosmid vectors is described in detail in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press (1989). Once ligated into an appropriate vector, two or more vectors containing different polyketide synthase gene clusters can be introduced into a suitable host cell. Regions of partial sequence homology shared by the gene clusters will promote processes which result in sequence reorganization resulting in a hybrid gene cluster. The novel hybrid gene cluster can then be screened for enhanced activities not found in the original gene clusters.

Thus, in one aspect, the invention relates to a method for producing a biologically active hybrid polypeptide using a nucleic acid of the invention and screening the polypeptide for an activity (e.g., enhanced activity) by:

(1) introducing at least a first polynucleotide (e.g., a nucleic acid of the invention) in operable linkage and a second polynucleotide in operable linkage, said at least first polynucleotide and second polynucleotide sharing at least one region of partial sequence homology, into a suitable host cell;

(2) growing the host cell under conditions which promote sequence reorganization resulting in a hybrid polynucleotide in operable linkage;

(3) expressing a hybrid polypeptide encoded by the hybrid polynucleotide;

(4) screening the hybrid polypeptide under conditions which promote identification of the desired biological activity (e.g., enhanced phospholipase activity); and

(5) isolating the a polynucleotide encoding the hybrid polypeptide.

Methods for screening for various enzyme activities are known to those of skill in the art and are discussed throughout the present specification. Such methods may be employed when isolating the polypeptides and polynucleotides of the invention.

In vivo reassortment can be focused on "inter-molecular" processes collectively referred to as "recombination." In bacteria it is generally viewed as a "RecA-dependent" phenomenon. The invention can rely on recombination processes of a host cell to recombine and re-assort sequences, or the cells' ability to mediate reductive processes to decrease the complexity of quasi-repeated sequences in the cell by deletion. This process of "reductive reassortment" occurs by an "intra-molecular", RecA-independent process. Thus, in one aspect of the invention, using the nucleic acids of the invention novel polynucleotides are generated by the process of reductive reassortment. The method involves the generation of constructs containing consecutive sequences (original encoding sequences), their insertion into an appropriate vector, and their subsequent introduction into an appropriate host cell. The reassortment of the individual molecular identities occurs by combinatorial processes between the consecutive sequences in the construct possessing regions of homology, or between quasi-repeated units. The reassortment process recombines and/or reduces the complexity and extent of the repeated sequences, and results in the production of novel molecular species.

Various treatments may be applied to enhance the rate of reassortment. These could include treatment with ultra-violet light, or DNA damaging chemicals, and/or the use of host cell lines displaying enhanced levels of "genetic instability". Thus the reassortment process may involve homologous recombination or the natural property of quasi-repeated sequences to direct their own evolution.

Repeated or "quasi-repeated" sequences play a role in genetic instability. "Quasi-repeats" are repeats that are not restricted to their original unit structure. Quasi-repeated units can be presented as an array of sequences in a construct; consecutive units of similar sequences. Once ligated, the junctions between the consecutive sequences become essentially invisible and the quasi-repetitive nature of the resulting construct is now

continuous at the molecular level. The deletion process the cell performs to reduce the complexity of the resulting construct operates between the quasi-repeated sequences. The quasi-repeated units provide a practically limitless repertoire of templates upon which slippage events can occur. The constructs containing the quasi-repeats thus effectively provide sufficient molecular elasticity that deletion (and potentially insertion) events can occur virtually anywhere within the quasi-repetitive units. When the quasi-repeated sequences are all ligated in the same orientation, for instance head to tail or vice versa, the cell cannot distinguish individual units. Consequently, the reductive process can occur throughout the sequences. In contrast, when for example, the units are presented head to head, rather than head to tail, the inversion delineates the endpoints of the adjacent unit so that deletion formation will favor the loss of discrete units. Thus, in one aspect of the invention, the sequences to be reassorted are in the same orientation. Random orientation of quasi-repeated sequences will result in the loss of reassortment efficiency, while consistent orientation of the sequences will offer the highest efficiency. However, while having fewer of the contiguous sequences in the same orientation decreases the efficiency, it may still provide sufficient elasticity for the effective recovery of novel molecules. Constructs can be made with the quasi-repeated sequences in the same orientation to allow higher efficiency.

Sequences can be assembled in a head to tail orientation using any of a variety of methods, including the following: a) Primers that include a poly-A head and poly-T tail which when made single-stranded would provide orientation can be utilized. This is accomplished by having the first few bases of the primers made from RNA and hence easily removed RNase H. b) Primers that include unique restriction cleavage sites can be utilized. Multiple sites, a battery of unique sequences, and repeated synthesis and ligation steps would be required. c) The inner few bases of the primer could be thiolated and an exonuclease used to produce properly tailed molecules.

The recovery of the re-assorted sequences relies on the identification of cloning vectors with a reduced repetitive index (RI). The re-assorted encoding sequences can then be recovered by amplification. The products are re-cloned and expressed. The recovery of cloning vectors with reduced RI can be affected by: 1) The use of vectors only stably maintained when the construct is reduced in complexity. 2)The physical recovery of shortened vectors by physical procedures. In this case, the cloning vector would be recovered using standard plasmid isolation procedures and size fractionated on either an agarose gel, or column with a low molecular weight cut off utilizing standard procedures. 3) The recovery

of vectors containing interrupted genes which can be selected when insert size decreases. 4) The use of direct selection techniques with an expression vector and the appropriate selection.

/ Encoding sequences (for example, genes) from related organisms may demonstrate a high degree of homology and encode quite diverse protein products. These types of sequences are particularly useful in the present invention as quasi-repeats. However, this process is not limited to such nearly identical repeats.

The following is an exemplary method of the invention. Encoding nucleic acid sequences (quasi-repeats) are derived from three (3) species, including a nucleic acid of the invention. Each sequence encodes a protein with a distinct set of properties, including an enzyme of the invention. Each of the sequences differs by a single or a few base pairs at a unique position in the sequence. The quasi-repeated sequences are separately or collectively amplified and ligated into random assemblies such that all possible permutations and combinations are available in the population of ligated molecules. The number of quasi-repeat units can be controlled by the assembly conditions. The average number of quasi-repeated units in a construct is defined as the repetitive index (RI). Once formed, the constructs may, or may not be size fractionated on an agarose gel according to published protocols, inserted into a cloning vector, and transfected into an appropriate host cell. The cells are then propagated and "reductive reassortment" is effected. The rate of the reductive reassortment process may be stimulated by the introduction of DNA damage if desired. Whether the reduction in RI is mediated by deletion formation between repeated sequences by an "intra-molecular" mechanism, or mediated by recombination-like events through "inter-molecular" mechanisms is immaterial. The end result is a reassortment of the molecules into all possible combinations. In one aspect, the method comprises the additional step of screening the library members of the shuffled pool to identify individual shuffled library members having the ability to bind or otherwise interact, or catalyze a particular reaction (e.g., such as catalytic domain of an enzyme) with a predetermined macromolecule, such as for example a proteinaceous receptor, an oligosaccharide, virion, or other predetermined compound or structure. The polypeptides, e.g., phospholipases, that are identified from such libraries can be used for various purposes, e.g., the industrial processes described herein and/or can be subjected to one or more additional cycles of shuffling and/or selection.

In another aspect, it is envisioned that prior to or during recombination or reassortment, polynucleotides generated by the method of the invention can be subjected to agents or processes which promote the introduction of mutations into the original

polynucleotides. The introduction of such mutations would increase the diversity of resulting hybrid polynucleotides and polypeptides encoded therefrom. The agents or processes which promote mutagenesis can include, but are not limited to: (+)-CC-1065, or a synthetic analog such as (+)-CC-1065-(N3-Adenine (See Sun and Hurley, (1992); an N-acetylated or deacetylated 4'-fluro-4-aminobiphenyl adduct capable of inhibiting DNA synthesis (See , for example, van de Poll et al. (1992)); or a N-acetylated or deacetylated 4-aminobiphenyl adduct capable of inhibiting DNA synthesis (See also, van de Poll et al. (1992), pp. 751-758); trivalent chromium, a trivalent chromium salt, a polycyclic aromatic hydrocarbon (PAH) DNA adduct capable of inhibiting DNA replication, such as 7-bromomethyl-benz[a]anthracene ("BMA"), tris(2,3-dibromopropyl)phosphate ("Tris-BP"), 1,2-dibromo-3-chloropropane ("DBCP"), 2-bromoacrolein (2BA), benzo[a]pyrene-7,8-dihydrodiol-9-10-epoxide ("BPDE"), a platinum(II) halogen salt, N-hydroxy-2-amino-3-methylimidazo[4,5-f]-quinoline ("N-hydroxy-IQ"), and N-hydroxy-2-amino-1-methyl-6-phenylimidazo[4,5-f]-pyridine ("N-hydroxy-PhIP"). Especially preferred means for slowing or halting PCR amplification consist of UV light (+)-CC-1065 and (+)-CC-1065-(N3-Adenine). Particularly encompassed means are DNA adducts or polynucleotides comprising the DNA adducts from the polynucleotides or polynucleotides pool, which can be released or removed by a process including heating the solution comprising the polynucleotides prior to further processing.

Screening Methodologies and "On-line" Monitoring Devices

In practicing the methods of the invention, a variety of apparatus and methodologies can be used to in conjunction with the polypeptides and nucleic acids of the invention, e.g., to screen polypeptides for phospholipase activity, to screen compounds as potential modulators of activity (e.g., potentiation or inhibition of enzyme activity), for antibodies that bind to a polypeptide of the invention, for nucleic acids that hybridize to a nucleic acid of the invention, and the like.

Immobilized Enzyme Solid Supports

The phospholipase enzymes, fragments thereof and nucleic acids that encode the enzymes and fragments can be affixed to a solid support. This is often economical and efficient in the use of the phospholipases in industrial processes. For example, a consortium or cocktail of phospholipase enzymes (or active fragments thereof), which are used in a specific chemical reaction, can be attached to a solid support and dunked into a process vat. The enzymatic reaction can occur. Then, the solid support can be taken out of the vat, along with the enzymes affixed thereto, for repeated use. In one embodiment of the invention, an

isolated nucleic acid of the invention is affixed to a solid support. In another embodiment of the invention, the solid support is selected from the group of a gel, a resin, a polymer, a ceramic, a glass, a microelectrode and any combination thereof.

For example, solid supports useful in this invention include gels. Some examples of gels include Sepharose, gelatin, glutaraldehyde, chitosan-treated glutaraldehyde, albumin-glutaraldehyde, chitosan-Xanthan, toyopearl gel (polymer gel), alginate, alginate-polylysine, carrageenan, agarose, glyoxyl agarose, magnetic agarose, dextran-agarose, poly(Carbamoyl Sulfonate) hydrogel, BSA-PEG hydrogel, phosphorylated polyvinyl alcohol (PVA), monoaminoethyl-N-aminoethyl (MANA), amino, or any combination thereof.

Another solid support useful in the present invention are resins or polymers. Some examples of resins or polymers include cellulose, acrylamide, nylon, rayon, polyester, anion-exchange resin, AMBERLITE™ XAD-7, AMBERLITE™ XAD-8, AMBERLITE™ IRA-94, AMBERLITE™ IRC-50, polyvinyl, polyacrylic, polymethacrylate, or any combination thereof.

Another type of solid support useful in the present invention is ceramic. Some examples include non-porous ceramic, porous ceramic, SiO_2, Al_2O_3. Another type of solid support useful in the present invention is glass. Some examples include non-porous glass, porous glass, aminopropyl glass or any combination thereof. Another type of solid support that can be used is a microelectrode. An example is a polyethyleneimine-coated magnetite. Graphitic particles can be used as a solid support.

Another example of a solid support is a cell, such as a red blood cell.

Methods of immobilization

There are many methods that would be known to one of skill in the art for immobilizing enzymes or fragments thereof, or nucleic acids, onto a solid support. Some examples of such methods include, e.g., electrostatic droplet generation, electrochemical means, via adsorption, via covalent binding, via cross-linking, via a chemical reaction or process, via encapsulation, via entrapment, via calcium alginate, or via poly (2-hydroxyethyl methacrylate). Like methods are described in Methods in Enzymology, Immobilized Enzymes and Cells, Part C. 1987. Academic Press. Edited by S. P. Colowick and N. O. Kaplan. Volume 136; and Immobilization of Enzymes and Cells. 1997. Humana Press. Edited by G. F. Bickerstaff. Series: Methods in Biotechnology, Edited by J. M. Walker.

Capillary Arrays

Capillary arrays, such as the GIGAMATRIX™, Diversa Corporation, San Diego, CA, can be used to in the methods of the invention. Nucleic acids or polypeptides of the invention can be immobilized to or applied to an array, including capillary arrays. Arrays can be used to screen for or monitor libraries of compositions (e.g., small molecules, antibodies, nucleic acids, etc.) for their ability to bind to or modulate the activity of a nucleic acid or a polypeptide of the invention. Capillary arrays provide another system for holding and screening samples. For example, a sample screening apparatus can include a plurality of capillaries formed into an array of adjacent capillaries, wherein each capillary comprises at least one wall defining a lumen for retaining a sample. The apparatus can further include interstitial material disposed between adjacent capillaries in the array, and one or more reference indicia formed within of the interstitial material. A capillary for screening a sample, wherein the capillary is adapted for being bound in an array of capillaries, can include a first wall defining a lumen for retaining the sample, and a second wall formed of a filtering material, for filtering excitation energy provided to the lumen to excite the sample.

A polypeptide or nucleic acid, e.g., a ligand, can be introduced into a first component into at least a portion of a capillary of a capillary array. Each capillary of the capillary array can comprise at least one wall defining a lumen for retaining the first component. An air bubble can be introduced into the capillary behind the first component. A second component can be introduced into the capillary, wherein the second component is separated from the first component by the air bubble. A sample of interest can be introduced as a first liquid labeled with a detectable particle into a capillary of a capillary array, wherein each capillary of the capillary array comprises at least one wall defining a lumen for retaining the first liquid and the detectable particle, and wherein the at least one wall is coated with a binding material for binding the detectable particle to the at least one wall. The method can further include removing the first liquid from the capillary tube, wherein the bound detectable particle is maintained within the capillary, and introducing a second liquid into the capillary tube.

The capillary array can include a plurality of individual capillaries comprising at least one outer wall defining a lumen. The outer wall of the capillary can be one or more walls fused together. Similarly, the wall can define a lumen that is cylindrical, square, hexagonal or any other geometric shape so long as the walls form a lumen for retention of a liquid or sample. The capillaries of the capillary array can be held together in close proximity to form a planar structure. The capillaries can be bound together, by being fused (e.g., where the capillaries are made of glass), glued, bonded, or clamped side-by-side. The

capillary array can be formed of any number of individual capillaries, for example, a range from 100 to 4,000,000 capillaries. A capillary array can form a microtiter plate having about 100,000 or more individual capillaries bound together.

Arrays, or "BioChips"

Nucleic acids or polypeptides of the invention can be immobilized to or applied to an array. Arrays can be used to screen for or monitor libraries of compositions (e.g., small molecules, antibodies, nucleic acids, etc.) for their ability to bind to or modulate the activity of a nucleic acid or a polypeptide of the invention. For example, in one aspect of the invention, a monitored parameter is transcript expression of a phospholipase gene. One or more, or, all the transcripts of a cell can be measured by hybridization of a sample comprising transcripts of the cell, or, nucleic acids representative of or complementary to transcripts of a cell, by hybridization to immobilized nucleic acids on an array, or "biochip." By using an "array" of nucleic acids on a microchip, some or all of the transcripts of a cell can be simultaneously quantified. Alternatively, arrays comprising genomic nucleic acid can also be used to determine the genotype of a newly engineered strain made by the methods of the invention. "Polypeptide arrays" can also be used to simultaneously quantify a plurality of proteins.

The present invention can be practiced with any known "array," also referred to as a "microarray" or "nucleic acid array" or "polypeptide array" or "antibody array" or "biochip," or variation thereof. Arrays are generically a plurality of "spots" or "target elements," each target element comprising a defined amount of one or more biological molecules, e.g., oligonucleotides, immobilized onto a defined area of a substrate surface for specific binding to a sample molecule, e.g., mRNA transcripts.

In practicing the methods of the invention, any known array and/or method of making and using arrays can be incorporated in whole or in part, or variations thereof, as described, for example, in U.S. Patent Nos. 6,277,628; 6,277,489; 6,261,776; 6,258,606; 6,054,270; 6,048,695; 6,045,996; 6,022,963; 6,013,440; 5,965,452; 5,959,098; 5,856,174; 5,830,645; 5,770,456; 5,632,957; 5,556,752; 5,143,854; 5,807,522; 5,800,992; 5,744,305; 5,700,637; 5,556,752; 5,434,049; see also, e.g., WO 99/51773; WO 99/09217; WO 97/46313; WO 96/17958; see also, e.g., Johnston (1998) Curr. Biol. 8:R171-R174; Schummer (1997) Biotechniques 23:1087-1092; Kern (1997) Biotechniques 23:120-124; Solinas-Toldo (1997) Genes, Chromosomes & Cancer 20:399-407; Bowtell (1999) Nature Genetics Supp. 21:25-

32. See also published U.S. patent applications Nos. 20010018642; 20010019827; 20010016322; 20010014449; 20010014448; 20010012537; 20010008765.

Antibodies and Antibody-based screening methods

The invention provides isolated or recombinant antibodies that specifically bind to a phospholipase of the invention. These antibodies can be used to isolate, identify or quantify the phospholipases of the invention or related polypeptides. These antibodies can be used to inhibit the activity of an enzyme of the invention. These antibodies can be used to isolated polypeptides related to those of the invention, e.g., related phospholipase enzymes. The antibodies can be used in immunoprecipitation, staining (e.g., FACS), immunoaffinity columns, and the like. If desired, nucleic acid sequences encoding for specific antigens can be generated by immunization followed by isolation of polypeptide or nucleic acid, amplification or cloning and immobilization of polypeptide onto an array of the invention. Alternatively, the methods of the invention can be used to modify the structure of an antibody produced by a cell to be modified, e.g., an antibody's affinity can be increased or decreased. Furthermore, the ability to make or modify antibodies can be a phenotype engineered into a cell by the methods of the invention.

Methods of immunization, producing and isolating antibodies (polyclonal and monoclonal) are known to those of skill in the art and described in the scientific and patent literature, see, e.g., Coligan, CURRENT PROTOCOLS IN IMMUNOLOGY, Wiley/Greene, NY (1991); Stites (eds.) BASIC AND CLINICAL IMMUNOLOGY (7th ed.) Lange Medical Publications, Los Altos, CA ("Stites"); Goding, MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE (2d ed.) Academic Press, New York, NY (1986); Kohler (1975) Nature 256:495; Harlow (1988) ANTIBODIES, A LABORATORY MANUAL, Cold Spring Harbor Publications, New York. Antibodies also can be generated in vitro, e.g., using recombinant antibody binding site expressing phage display libraries, in addition to the traditional in vivo methods using animals. See, e.g., Hoogenboom (1997) Trends Biotechnol. 15:62-70; Katz (1997) Annu. Rev. Biophys. Biomol. Struct. 26:27-45.

The polypeptides can be used to generate antibodies which bind specifically to the polypeptides of the invention. The resulting antibodies may be used in immunoaffinity chromatography procedures to isolate or purify the polypeptide or to determine whether the polypeptide is present in a biological sample. In such procedures, a protein preparation, such as an extract, or a biological sample is contacted with an antibody capable of specifically binding to one of the polypeptides of the invention.

In immunoaffinity procedures, the antibody is attached to a solid support, such as a bead or other column matrix. The protein preparation is placed in contact with the antibody under conditions in which the antibody specifically binds to one of the polypeptides of the invention. After a wash to remove non-specifically bound proteins, the specifically bound polypeptides are eluted.

The ability of proteins in a biological sample to bind to the antibody may be determined using any of a variety of procedures familiar to those skilled in the art. For example, binding may be determined by labeling the antibody with a detectable label such as a fluorescent agent, an enzymatic label, or a radioisotope. Alternatively, binding of the antibody to the sample may be detected using a secondary antibody having such a detectable label thereon. Particular assays include ELISA assays, sandwich assays, radioimmunoassays, and Western Blots.

Polyclonal antibodies generated against the polypeptides of the invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, for example, a nonhuman. The antibody so obtained will then bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies which may bind to the whole native polypeptide. Such antibodies can then be used to isolate the polypeptide from cells expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique, the trioma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (see, e.g., Cole (1985) in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (see, e.g., U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to the polypeptides of the invention. Alternatively, transgenic mice may be used to express humanized antibodies to these polypeptides or fragments thereof.

Antibodies generated against the polypeptides of the invention may be used in screening for similar polypeptides from other organisms and samples. In such techniques, polypeptides from the organism are contacted with the antibody and those polypeptides which specifically bind the antibody are detected. Any of the procedures described above may be used to detect antibody binding.

Kits

The invention provides kits comprising the compositions, e.g., nucleic acids, expression cassettes, vectors, cells, polypeptides (e.g., phospholipases) and/or antibodies of the invention. The kits also can contain instructional material teaching the methodologies and industrial uses of the invention, as described herein.

Industrial and Medical Uses of the Enzymes of the Invention

The invention provides many industrial uses and medical applications for the enzymes of the invention, e.g., phospholipases A, B, C and D, including converting a non-hydratable phospholipid to a hydratable form, oil degumming, processing of oils from plants, fish, algae and the like, to name just a few applications. Methods of using phospholipase enzymes in industrial applications are well known in the art. For example, the phospholipases and methods of the invention can be used for the processing of fats and oils as described, e.g., in JP Patent Application Publication H6-306386, describing converting phospholipids present in the oils and fats into water-soluble substances containing phosphoric acid groups.

Phospholipases of the invention can be used to process plant oils and phospholipids such as those derived from or isolated from soy, canola, palm, cottonseed, corn, palm kernel, coconut, peanut, sesame, sunflower. Phospholipases of the invention can be used to process essential oils, e.g., those from fruit seed oils, e.g., grapeseed, apricot, borage, etc. Phospholipases of the invention can be used to process oils and phospholipids in different forms, including crude forms, degummed, gums, wash water, clay, silica, soapstock, and the like. The phospholipids of the invention can be used to process high phosphorous oils, fish oils, animal oils, plant oils, algae oils and the like. In any aspect of the invention, any time a phospholipase C can be used, an alternative comprises use of a phospholipase D of the invention and a phosphatase (e.g., using a PLD/ phosphatase combination to improve yield in a high phosphorus oil, such as a soy bean oil).

Phospholipases of the invention can be used to process and make edible oils, biodiesel oils, liposomes for pharmaceuticals and cosmetics, structured phospholipids and structured lipids. Phospholipases of the invention can be used in oil extraction.

Phospholipases of the invention can be used to process and make various soaps.

Caustic refining

In one exemplary process of the invention, phospholipases are used as caustic refining aids. More particularly a PLC or PLD and a phosphatase are used in the processes as adrop-in, either before, during, or after a caustic neutralization refining process (either continuous or batch refining. The amount of enzyme added may vary according to the process. The water level used in the process should be low, e.g., about 0.5 to 5%. Alternatively, caustic is be added to the process multiple times. In addition, the process may be performed at different temperatures (25°C to 70°C), with different acids orcaustics, and at varying pH (4-12). Acids that may be used in a caustic refining process include, but are not limited to, phosphoric, citric, ascorbic, sulfuric, fumaric, maleic, hydrochloric and/or acetic acids. Acids are used to hydrate non-hydratable phospholipids. Caustics that may be used include, but are not limited to, KOH- and NaOH. Caustics are used to neutralize free fatty acids. Alternatively, phospholipases, or more particularly a PLC or a PLD and a phosphatase, are used for purification of phytosterols from the gum/soapstock.

An alternate embodiment of the invention to add the phospholipase before caustic refining is to express the phospholipase in a plant. In another embodiment, the phospholipase is added during crushing of the plant, seeds or other plant part. Alternatively, the phospholipase is added following crushing, but prior to refining (i.e. in holding vessels). In addition, phospholipase is added as a refining pre-treatment, either with or without acid.

Another embodiment of the invention, already described, is to add the phospholipase during a caustic refining process. In this process, the levels of acid and caustic are varied depending on the level of phosphorous and the level of free fatty acids. In addition, broad temperature and pH ranges are used in the process, dependent upon the type of enzyme used.

In another embodiment of the invention, the phospholipase is added after caustic refining (Fig. 9). In one instance, the phospholipase is added in an intense mixer or in a retention mixer, prior to separation. Alternatively, the phospholipase is added following the heat step. In another embodiment, the phospholipase is added in the centrifugation step. In an additional embodiment, the phospholipase is added to the soapstock. Alternatively, the phospholipase is added to the washwater. In another instance, the phospholipase is added during the bleaching and/or deodorizing steps.

Oil degumming and vegetable oil processing

The phospholipases of the invention can be used in various vegetable oil processing steps, such as in vegetable oil extraction, particularly, in the removal of

"phospholipid gums" in a process called "oil degumming," as described above. The invention provides methods for processing vegetable oils from various sources, such as soybeans, rapeseed, peanuts and other nuts, sesame, sunflower, palm and corn. The methods can used in conjunction with processes based on extraction with as hexane, with subsequent refining of the crude extracts to edible oils, including use of the methods and enzymes of the invention. The first step in the refining sequence is the so-called "degumming" process, which serves to separate phosphatides by the addition of water. The material precipitated by degumming is separated and further processed to mixtures of lecithins. The commercial lecithins, such as soybean lecithin and sunflower lecithin, are semi-solid or very viscous materials. They consist of a mixture of polar lipids, mainly phospholipids, and oil, mainly triglycerides.

The phospholipases of the invention can be used in any "degumming" procedure, including water degumming, ALCON oil degumming (e.g., for soybeans), safinco degumming, "super degumming," UF degumming, TOP degumming, uni-degumming, dry degumming and ENZYMAX™ degumming. See, e.g., U.S. Patent Nos. 6,355,693; 6,162,623; 6,103,505; 6,001,640; 5,558,781; 5,264,367. Various "degumming" procedures incorporated by the methods of the invention are described in Bockisch, M. (1998) In Fats and Oils Handbook, The extraction of Vegetable Oils (Chapter 5), 345-445, AOCS Press, Champaign, Illinois. The phospholipases of the invention can be used in the industrial application of enzymatic degumming of triglyceride oils as described, e.g., in EP 513 709.

The phospholipases of the invention can be used in the industrial application of enzymatic degumming as described, e.g., in CA 1102795, which describes a method of isolating polar lipids from cereal lipids by the addition of at least 50% by weight of water. This method is a modified degumming in the sense that it utilizes the principle of adding water to a crude oil mixture.

In one aspect, the invention provides enzymatic processes comprising use of phospholipases of the invention (e.g., a PLC) comprising hydrolysis of hydrated phospholipids in oil at a temperature of about 20°C to 40°C, at an alkaline pH, e.g., a pH of about pH 8 to pH 10, using a reaction time of about 3 to 10 minutes. This can result in less than 10 ppm final oil phosphorus levels. The invention also provides enzymatic processes comprising use of phospholipases of the invention (e.g., a PLC) comprising hydrolysis of hydratable and non-hydratable phospholipids in oil at a temperature of about 50°C to 60°C, at a pH slightly below neutral, e.g., of about pH 5 to pH 6.5, using a reaction time of about 30 to 60 minutes. This can result in less than 10 ppm final oil phosphorus levels.

In one aspect, the invention provides enzymatic processes that utilize a phospholipase C enzyme to hydrolyze a glyceryl phosphoester bond and thereby enable the return of the diacylglyceride portion of phospholipids back to the oil, e.g., a vegetable, fish or algae oil (a "phospholipase C (PLC) caustic refining aid"); and, reduce the phospholipid content in a degumming step to levels low enough for high phosphorous oils to be physically refined (a "phospholipase C (PLC) degumming aid"). The two approaches can generate different values and have different target applications.

In various exemplary processes of the invention, a number of distinct steps compose the degumming process preceding the core bleaching and deodorization refining processes. These steps include heating, mixing, holding, separating and drying. Following the heating step, water and often acid are added and mixed to allow the insoluble phospholipid "gum" to agglomerate into particles which may be separated. While water separates many of the phosphatides in degumming, portions of the phospholipids are non-hydratable phosphatides (NHPs) present as calcium or magnesium salts. Degumming processes address these NHPs by the addition of acid. Following the hydration of phospholipids, the oil is mixed, held and separated by centrifugation. Finally, the oil is dried and stored, shipped or refined, as illustrated, e.g., in Figure 6. The resulting gums are either processed further for lecithin products or added back into the meal.

In various exemplary processes of the invention phosphorous levels are reduced low enough for physical refining. The separation process can result in potentially higher yield losses than caustic refining. Additionally, degumming processes may generate waste products that may not be sold as commercial lecithin, see, e.g., Figure 7 for an exemplary degumming process for physically refined oils. Therefore, these processes have not achieved a significant share of the market and caustic refining processes continue to dominate the industry for soy, canola and sunflower. Note however, that a phospholipase C enzyme employed in a special degumming process would decrease gum formation and return the diglyceride portion of the phospholipid back to the oil.

In one aspect, a phospholipase C enzyme of the invention hydrolyzes a phosphatide at a glyceryl phosphoester bond to generate a diglyceride and water-soluble phosphate compound. The hydrolyzed phosphatide moves to the aqueous phase, leaving the diglyceride in the oil phase, as illustrated in Figure 8. One objective of the PLC "Caustic Refining Aid" is to convert the phospholipid gums formed during neutralization into a diacylglyceride that will migrate back into the oil phase. In contrast, one objective of the

"PLC Degumming Aid" is to reduce the phospholipids in crude oil to a phosphorous equivalent of less than 10 parts per million (ppm).

In one aspect, a phospholipase C enzyme of the invention will hydrolyze the phosphatide from both hydratable and non-hydratable phospholipids in neutralized crude and degummed oils before bleaching and deodorizing. The target enzyme can be applied as a drop-in product in the existing caustic neutralization process, as illustrated in Figure 9. In this aspect, the enzyme will not be required to withstand extreme pH levels if it is added after the addition of caustic.

In one aspect, a phospholipase of the invention enables phosphorous to be removed to the low levels acceptable in physical refining. In one aspect, a PLC of the invention will hydrolyze the phosphatide from both hydratable and non-hydratable phospholipids in crude oils before bleaching and deodorizing. The target enzyme can be applied as a drop-in product in the existing degumming operation, see, e.g., Figure 10. Given sub-optimal mixing in commercial equipment, it is likely that acid will be required to bring the non-hydratable phospholipids in contact with the enzyme at the oil/water interface. Therefore, in one aspect, an acid-stable PLC of the invention is used.

In one aspect, a PLC Degumming Aid process of the invention can eliminate losses in one, or all three, areas noted in Table 2. Losses associated in a PLC process can be estimated to be about 0.8% versus 5.2% on a mass basis due to removal of the phosphatide.

Table 2: Losses Addressed by PLC Products

	Caustic Refining Aid	Degumming Aid
1) Oil lost in gum formation & separation 2.1%	X	X
2) Saponified oil in caustic addition 3.1%		X
3) Oil trapped in clay in bleaching* <1.0%	X	X
Total Yield Loss ~5.2%	~2.1%	~5.2%

Additional potential benefits of this process of the invention include the following:

- Reduced adsorbents – less adsorbents required with lower (< 5ppm) phosphorous
- Lower chemical usage – less chemical and processing costs associated with hydration of non-hydratable phospholipids
- Lower waste generation – less water required to remove phosphorous from oil

Oils processed (e.g., "degummed") by the methods of the invention include plant oilseeds, e.g., soybean oil, rapeseed oil and sunflower oil. In one aspect, the "PLC Caustic Refining Aid" of the invention can save 1.2% over existing caustic refining processes. The refining aid application addresses soy oil that has been degummed for lecithin and these are also excluded from the value/load calculations.

Performance targets of the processes of the invention can vary according to the applications and more specifically to the point of enzyme addition, see Table 3.

Table 3: Performance Targets by Application

	Caustic Refining Aid	Degumming Aid
Incoming Oil Phosphorous Levels	<200 ppm*	600-1,400 ppm
Final Oil Phosphorous Levels	<10 ppm†	<10 ppm
Hydratable & Non-hydratable gums	Yes	Yes
Residence Time	3-10 minutes	30 minutes‡
Liquid Formulation	Yes	Yes
Target pH	8-10‡‡‡	5.0-5.5‡‡
Target Temperature	20-40°C	~50-60°C
Water Content	<5%	1-1.25%
Enzyme Formulation Purity	No lipase/protease‡	No lipase/protease
Other Key Requirements	Removal of Fe	Removal of Fe

**Water degummed oil*
†Target levels achieved in upstream caustic neutralization step but must be maintained
‡1-2 hours existing
‡‡Acid degumming will require an enzyme that is stable in much more acidic conditions: pH at 2.3 for citric acid at 5%. (~Roehm USPN 6,001,640).
‡‡‡The pH of neutralized oil is NOT neutral. Testing at POS indicates that the pH will be in the alkaline range from 6.5-10 (December 9, 2002). Typical pH range needs to be determined.

Other processes that can be used with a phospholipase of the invention, e.g., a phospholipase A_1 can convert non-hydratable native phospholipids to a hydratable form. In one aspect, the enzyme is sensitive to heat. This may be desirable, since heating the oil can destroy the enzyme. However, the degumming reaction must be adjusted to pH 4-5 and 60°C to accommodate this enzyme. At 300 Units/kg oil saturation dosage, this exemplary process is successful at taking previously water-degummed oil phosphorous content down to ≤10 ppm P. Advantages can be decreased H_20 content and resultant savings in usage, handling and waste. Table 4 lists exemplary applications for industrial uses for enzymes of the invention:

Table 4: Exemplary Application

	Caustic Refining Aid	Degumming Aid
Soy oil w/ lecithin production	X	
Chemical refined soy oil, Sunflower oil, Canola oil	X	X

Low phosphatide oils (e.g. palm)		X

In addition to these various "degumming" processes, the phospholipases of the invention can be used in any vegetable oil processing step. For example, phospholipase enzymes of the invention can be used in place of PLA, e.g., phospholipase A2, in any vegetable oil processing step. Oils that are "processed" or "degummed" in the methods of the invention include soybean oils, rapeseed oils, corn oils, oil from palm kernels, canola oils, sunflower oils, sesame oils, peanut oils, and the like. The main products from this process include triglycerides.

In one exemplary process, when the enzyme is added to and reacted with a crude oil, the amount of phospholipase employed is about 10-10,000 units, or, alternatively, about, 100-2,000 units, per 1 kg of crude oil. The enzyme treatment is conducted for 5 min to 10 hours at a temperature of 30°C to 90°C, or, alternatively, about, 40°C to 70°C. The conditions may vary depending on the optimum temperature of the enzyme. The amount of water added to dissolve the enzyme is 5-1,000 wt. parts per 100 wt. parts of crude oil, or, alternatively, about, 10 to 200 wt. parts per 100 wt. parts of crude oil.

Upon completion of such enzyme treatment, the enzyme liquid is separated with an appropriate means such as a centrifugal separator and the processed oil is obtained. Phosphorus-containing compounds produced by enzyme decomposition of gummy substances in such a process are practically all transferred into the aqueous phase and removed from the oil phase. Upon completion of the enzyme treatment, if necessary, the processed oil can be additionally washed with water or organic or inorganic acid such as, e.g., acetic acid, phosphoric acid, succinic acid, and the like, or with salt solutions.

In one exemplary process for ultra-filtration degumming, the enzyme is bound to a filter or the enzyme is added to an oil prior to filtration or the enzyme is used to periodically clean filters.

In one exemplary process for a phospholipase-mediated physical refining aid, water and enzyme are added to crude oil. In one aspect, a PLC or a PLD and a phosphatase are used in the process. In phospholipase-mediated physical refining, the water level can be low, i.e. 0.5 – 5% and the process time should be short (less than 2 hours, or, less than 60 minutes, or, less than 30 minutes, or, less than 15 minutes, or, less than 5 minutes). The process can be run at different temperatures (25°C to 70°C), using different acids and/or caustics, at different pHs (e.g., 3-10).

In alternate aspects, water degumming is performed first to collect lecithin by centrifugation and then PLC or PLC and PLA is added to remove non-hydratable phospholipids (the process should be performed under low water concentration). In another aspect, water degumming of crude oil to less than 10 ppm (edible oils) and subsequent physical refining (less than 50 ppm for biodiesel) is performed. In one aspect, an emulsifier is added and/or the crude oil is subjected to an intense mixer to promote mixing. Alternatively, an emulsion-breaker is added and/or the crude oil is heated to promote separation of the aqueous phase. In another aspect, an acid is added to promote hydration of non-hydratable phospholipids. Additionally, phospholipases can be used to mediate purification of phytosterols from the gum/soapstock.

The enzymes of the invention can be used in any oil processing method, e.g., degumming or equivalent processes. For example, the enzymes of the invention can be used in processes as described in U.S. Patent Nos. 5,558,781; 5,264,367; 6,001,640. The process described in USPN 5,558,781 uses either phospholipase A1, A2 or B, essentially breaking down lecithin in the oil that behaves as an emulsifier.

The enzymes and methods of the invention can be used in processes for the reduction of phosphorus-containing components in edible oils comprising a high amount of non-hydratable phosphorus by using of a phospholipase of the invention, e.g., a polypeptide having a phospholipase A and/or B activity, as described, e.g., in EP Patent Number: EP 0869167. In one aspect, the edible oil is a crude oil, a so-called "non-degummed oil." In one aspect, the method treat a non-degummed oil, including pressed oils or extracted oils, or a mixture thereof, from, e.g., rapeseed, soybean, sesame, peanut, corn or sunflower. The phosphatide content in a crude oil can vary from 0.5 to 3% w/w corresponding to a phosphorus content in the range of 200 to 1200 ppm, or, in the range of 250 to 1200 ppm. Apart from the phosphatides, the crude oil can also contains small concentrations of carbohydrates, sugar compounds and metal/phosphatide acid complexes of Ca, Mg and Fe. In one aspect, the process comprises treatment of a phospholipid or lysophospholipid with the phospholipase of the invention so as to hydrolyze fatty acyl groups. In one aspect, the phospholipid or lysophospholipid comprises lecithin or lysolecithin. In one aspect of the process the edible oil has a phosphorus content from between about 50 to 250 ppm, and the process comprises treating the oil with a phospholipase of the invention so as to hydrolyze a major part of the phospholipid and separating an aqueous phase containing the hydrolyzed phospholipid from the oil. In one aspect, prior to the enzymatic degumming process the oil is water-degummed. In one aspect, the methods provide for the production of an animal feed

comprising mixing the phospholipase of the invention with feed substances and at least one phospholipid.

The enzymes and methods of the invention can be used in processes of oil degumming as described, e.g., in WO 98/18912. The phospholipases of the invention can be used to reduce the content of phospholipid in an edible oil. The process can comprise treating the oil with a phospholipase of the invention to hydrolyze a major part of the phospholipid and separating an aqueous phase containing the hydrolyzed phospholipid from the oil. This process is applicable to the purification of any edible oil, which contains a phospholipid, e.g. vegetable oils, such as soybean oil, rapeseed oil and sunflower oil, fish oils, algae and animal oils and the like. Prior to the enzymatic treatment, the vegetable oil is preferably pretreated to remove slime (mucilage), e.g. by wet refining. The oil can contain 50-250 ppm of phosphorus as phospholipid at the start of the treatment with phospholipase, and the process of the invention can reduce this value to below 5-10 ppm.

The enzymes of the invention can be used in processes as described in JP Application No.: H5-132283, filed April 25, 1993, which comprises a process for the purification of oils and fats comprising a step of converting phospholipids present in the oils and fats into water-soluble substances containing phosphoric acid groups and removing them as water-soluble substances. An enzyme action is used for the conversion into water-soluble substances. An enzyme having a phospholipase C activity is preferably used as the enzyme.

The enzymes of the invention can be used in processes as described as the "Organic Refining Process," (ORP) (IPH, Omaha, NE) which is a method of refining seed oils. ORP may have advantages over traditional chemical refining, including improved refined oil yield, value added co-products, reduced capital costs and lower environmental costs.

The enzymes of the invention can be used in processes for the treatment of an oil or fat, animal or vegetal, raw, semi-processed or refined, comprising adding to such oil or fat at least one enzyme of the invention that allows hydrolyzing and/or depolymerizing the non-glyceridic compounds contained in the oil, as described, e.g., in EP Application number: 82870032.8. Exemplary methods of the invention for hydrolysis and/or depolymerization of non-glyceridic compounds in oils are:

1) The addition and mixture in oils and fats of an enzyme of the invention or enzyme complexes previously dissolved in a small quantity of appropriate solvent (for example water). A certain number of solvents are possible, but a non-toxic and suitable solvent for the enzyme is chosen. This addition may be done in processes

with successive loads, as well as in continuous processes. The quantity of enzyme(s) necessary to be added to oils and fats, according to this process, may range, depending on the enzymes and the products to be processed, from 20 to 400 ppm, i.e., from 0.02 kg to 0.4 kg of enzyme for 1000 kg of oil or fat, and preferably from 20 to 100 ppm, i.e., from 0.02 to 0.1 kg of enzyme for 1000 kg of oil, these values being understood to be for concentrated enzymes, i.e., without diluent or solvent.

2) Passage of the oil or fat through a fixed or insoluble filtering bed of enzyme(s) of the invention on solid or semi-solid supports, preferably presenting a porous or fibrous structure. In this technique, the enzymes are trapped in the micro-cavities of the porous or fibrous structure of the supports. These consist, for example, of resins or synthetic polymers, cellulose carbonates, gels such as agarose, filaments of polymers or copolymers with porous structure, trapping small droplets of enzyme in solution in their cavities. Concerning the enzyme concentration, it is possible to go up to the saturation of the supports.

3) Dispersion of the oils and fats in the form of fine droplets, in a diluted enzymatic solution, preferably containing 0.2 to 4% in volume of an enzyme of the invention. This technique is described, e.g., in Belgian patent No. 595,219. A cylindrical column with a height of several meters, with conical lid, is filled with a diluted enzymatic solution. For this purpose, a solvent that is non-toxic and non-miscible in the oil or fat to be processed, preferably water, is chosen. The bottom of the column is equipped with a distribution system in which the oil or fat is continuously injected in an extremely divided form (approximately 10,000 flux per m^2). Thus an infinite number of droplets of oil or fat are formed, which slowly rise in the solution of enzymes and meet at the surface, to be evacuated continuously at the top of the conical lid of the reactor.

Palm oil can be pre-treated before treatment with an enzyme of the invention. For example, about 30 kg of raw palm oil is heated to +50°C. 1% solutions were prepared in distilled water with cellulases and pectinases. 600 g of each of these was added to aqueous solutions of the oil under strong agitation for a few minutes. The oil is then kept at +50°C under moderate agitation, for a total reaction time of two hours. Then, temperature is raised to +90°C to deactivate the enzymes and prepare the mixture for filtration and further processing. The oil is dried under vacuum and filtered with a filtering aid.

The enzymes of the invention can be used in processes as described in EP patent EP 0 513 709 B2. For example, the invention provides a process for the reduction of

the content process for the reduction of the content of phosphorus-containing components in animal and vegetable oils by enzymatic decomposition using a phospholipase of the invention. A predemucilaginated animal and vegetable oil with a phosphorus content of 50 to 250 ppm is agitated with an organic carboxylic acid and the pH value of the resulting mixture set to pH 4 to pH 6, an enzyme solution which contains phospholipase A_1, A_2, or B of the invention is added to the mixture in a mixing vessel under turbulent stirring and with the formation of fine droplets, where an emulsion with 0.5 to 5 % by weight relative to the oil is formed, said emulsion being conducted through at least one subsequent reaction vessel under turbulent motion during a reaction time of 0.1 to 10 hours at temperatures in the range of 20 to 80° C and where the treated oil, after separation of the aqueous solution, has a phosphorus content under 5 ppm.

The organic refining process is applicable to both crude and degummed oil. The process uses inline addition of an organic acid under controlled process conditions, in conjunction with conventional centrifugal separation. The water separated naturally from the vegetable oil phospholipids ("VOP") is recycled and reused. The total water usage can be substantially reduced as a result of the Organic Refining Process.

The phospholipases and methods of the invention can also be used in the enzymatic treatment of edible oils, as described, e.g., in U.S. Patent No. 6,162,623. In this exemplary methods, the invention provides an amphiphilic enzyme. It can be immobilized, e.g., by preparing an emulsion containing a continuous hydrophobic phase and a dispersed aqueous phase containing the enzyme and a carrier for the enzyme and removing water from the dispersed phase until this phase turns into solid enzyme coated particles. The enzyme can be a lipase. The immobilized lipase can be used for reactions catalyzed by lipase such as interesterification of mono-, di- or triglycerides, de-acidification of a triglyceride oil, or removal of phospholipids from a triglyceride oil when the lipase is a phospholipase. The aqueous phase may contain a fermentation liquid, an edible triglyceride oil may be the hydrophobic phase, and carriers include sugars, starch, dextran, water soluble cellulose derivatives and fermentation residues. This exemplary method can be used to process triglycerides, diglycerides, monoglycerides, glycerol, phospholipids or fatty acids, which may be in the hydrophobic phase. In one aspect, the process for the removal of phospholipids from triglyceride oil comprising mixing a triglyceride oil containing phospholipids with a preparation containing a phospholipase of the invention; hydrolyzing the phospholipids to lysophospholipid; separating the hydrolyzed phospholipids from the oil, wherein the phospholipase is an immobilized phospholipase.

The phospholipases and methods of the invention can also be used in the enzymatic treatment of edible oils, as described, e.g., in U.S. Patent No. 6,127,137. This exemplary method hydrolyzes both fatty acyl groups in intact phospholipid. The phospholipase of the invention used in this methods has no lipase activity and is active at very low pH. These properties make it very suitable for use in oil degumming, as enzymatic and alkaline hydrolysis (saponification) of the oil can both be suppressed. In one aspect, the invention provides a process for hydrolyzing fatty acyl groups in a phospholipid or lysophospholipid comprising treating the phospholipid or lysophospholipid with the phospholipase that hydrolyzes both fatty acyl groups in a phospholipid and is essentially free of lipase activity. In one aspect, the phospholipase of the invention has a temperature optimum at about 50°C, measured at pH 3 to pH 4 for 10 minutes, and a pH optimum of about pH 3, measured at 40°C for about 10 minutes. In one aspect, the phospholipid or lysophospholipid comprises lecithin or lysolecithin. In one aspect, after hydrolyzing a major part of the phospholipid, an aqueous phase containing the hydrolyzed phospholipid is separated from the oil. In one aspect, the invention provides a process for removing phospholipid from an edible oil, comprising treating the oil at pH 1.5 to 3 with a dispersion of an aqueous solution of the phospholipase of the invention, and separating an aqueous phase containing the hydrolyzed phospholipid from the oil. In one aspect, the oil is treated to remove mucilage prior to the treatment with the phospholipase. In one aspect, the oil prior to the treatment with the phospholipase contains the phospholipid in an amount corresponding to 50 to 250 ppm of phosphorus. In one aspect, the treatment with phospholipase is done at 30°C to 45°C for 1 to 12 hours at a phospholipase dosage of 0.1 to 10 mg/l in the presence of 0.5 to 5% of water.

The phospholipases and methods of the invention can also be used in the enzymatic treatment of edible oils, as described, e.g., in U.S. Patent No. 6,025,171. In this exemplary methods, enzymes of the invention are immobilized by preparing an emulsion containing a continuous hydrophobic phase, such as a triglyceride oil, and a dispersed aqueous phase containing an amphiphilic enzyme, such as lipase or a phospholipase of the invention, and carrier material that is partly dissolved and partly undissolved in the aqueous phase, and removing water from the aqueous phase until the phase turns into solid enzyme coated carrier particles. The undissolved part of the carrier material may be a material that is insoluble in water and oil, or a water soluble material in undissolved form because the aqueous phase is already saturated with the water soluble material. The aqueous phase may be formed with a crude lipase fermentation liquid containing fermentation residues and

biomass that can serve as carrier materials. Immobilized lipase is useful for ester re-arrangement and de-acidification in oils. After a reaction, the immobilized enzyme can be regenerated for a subsequent reaction by adding water to obtain partial dissolution of the carrier, and with the resultant enzyme and carrier-containing aqueous phase dispersed in a hydrophobic phase evaporating water to again form enzyme coated carrier particles.

The phospholipases and methods of the invention can also be used in the enzymatic treatment of edible oils, as described, e.g., in U.S. Patent No. 6,143,545. This exemplary method is used for reducing the content of phosphorous containing components in an edible oil comprising a high amount of non-hydratable phosphorus content using a phospholipase of the invention. In one aspect, the method is used to reduce the content of phosphorus containing components in an edible oil having a non-hydratable phosphorus content of at least 50 ppm measured by pre-treating the edible oil, at 60°C, by addition of a solution comprising citric acid monohydrate in water (added water vs. oil equals 4.8% w/w; (citric acid) in water phase = 106 mM, in water/oil emulsion = 4.6 mM) for 30 minutes; transferring 10 ml of the pre-treated water in oil emulsion to a tube; heating the emulsion in a boiling water bath for 30 minutes; centrifuging at 5000 rpm for 10 minutes, transferring about 8 ml of the upper (oil) phase to a new tube and leaving it to settle for 24 hours; and drawing 2 g from the upper clear phase for measurement of the non-hydratable phosphorus content (ppm) in the edible oil. The method also can comprise contacting an oil at a pH from about pH 5 to 8 with an aqueous solution of a phospholipase A or B of the invention (e.g., PLA1, PLA2, or a PLB), which solution is emulsified in the oil until the phosphorus content of the oil is reduced to less than 11 ppm, and then separating the aqueous phase from the treated oil.

The phospholipases and methods of the invention can also be used in the enzymatic treatment of edible oils, as described, e.g., in U.S. Patent No. 5,532,163. The invention provides processes for the refining of oil and fat by which phospholipids in the oil and fat to be treated can be decomposed and removed efficiently. In one aspect, the invention provides a process for the refining of oil and fat which comprises reacting, in an emulsion, the oil and fat with an enzyme of the invention, e.g., an enzyme having an activity to decompose glycerol-fatty acid ester bonds in glycerophospholipids (e.g., a PLA2 of the invention); and another process in which the enzyme-treated oil and fat is washed with water or an acidic aqueous solution. In one aspect, the acidic aqueous solution to be used in the washing step is a solution of at least one acid, e.g., citric acid, acetic acid, phosphoric acid and salts thereof. In one aspect, the emulsified condition is formed using 30 weight parts or more of water per 100 weight parts of the oil and fat. Since oil and fat can be purified

without employing the conventional alkali refining step, generation of washing waste water and industrial waste can be reduced. In addition, the recovery yield of oil is improved because loss of neutral oil and fat due to their inclusion in these wastes does not occur in the inventive process. In one aspect, the invention provides a process for refining oil and fat containing about 100 to 10,000 ppm of phospholipids which comprises: reacting, in an emulsified condition, said oil and fat with an enzyme of the invention having activity to decompose glycerol-fatty acid ester bonds in glycerophospholipids. In one aspect, the invention provides processes for refining oil and fat containing about 100 to 10,000 ppm of phospholipids which comprises reacting, in an emulsified condition, oil and fat with an enzyme of the invention having activity to decompose glycerol-fatty acid ester bonds in glycerophospholipids; and subsequently washing the treated oil and fat with a washing water.

The phospholipases and methods of the invention can also be used in the enzymatic treatment of edible oils, as described, e.g., in U.S. Patent No. 5,264,367. The content of phosphorus-containing components and the iron content of an edible vegetable or animal oil, such as an oil, e.g., soybean oil, which has been wet-refined to remove mucilage, are reduced by enzymatic decomposition by contacting the oil with an aqueous solution of an enzyme of the invention, e.g., a phospholipase A1, A2, or B, and then separating the aqueous phase from the treated oil. In one aspect, the invention provides an enzymatic method for decreasing the content of phosphorus- and iron-containing components in oils, which have been refined to remove mucilage. An oil, which has been refined to remove mucilage, can be treated with an enzyme of the invention, e.g., phospholipase C, A1, A2, or B. Phosphorus contents below 5 ppm and iron contents below 1 ppm can be achieved. The low iron content can be advantageous for the stability of the oil.

The phospholipases and methods of the invention can also be used for preparing transesterified oils, as described, e.g., in U.S. Patent No. 5,288,619. The invention provides methods for enzymatic transesterification for preparing a margarine oil having both low trans- acid and low intermediate chain fatty acid content. The method includes the steps of providing a transesterification reaction mixture containing a stearic acid source material and an edible liquid vegetable oil, transesterifying the stearic acid source material and the vegetable oil using a 1-, 3- positionally specific lipase, and then finally hydrogenating the fatty acid mixture to provide a recycle stearic acid source material for a recyclic reaction with the vegetable oil. The invention also provides a counter- current method for preparing a transesterified oil. The method includes the steps of providing a transesterification reaction zone containing a 1-, 3-positionally specific lipase, introducing a vegetable oil into the

transesterification zone, introducing a stearic acid source material, conducting a supercritical gas or subcritical liquefied gas counter- current fluid, carrying out a transesterification reaction of the triglyceride stream with the stearic acid or stearic acid monoester stream in the reaction zone, withdrawing a transesterified triglyceride margarine oil stream, withdrawing a counter-current fluid phase, hydrogenating the transesterified stearic acid or stearic acid monoester to provide a hydrogenated recycle stearic acid source material, and introducing the hydrogenated recycle stearic acid source material into the reaction zone.

In one aspect, the highly unsaturated phospholipid compound may be converted into a triglyceride by appropriate use of a phospholipase C of the invention to remove the phosphate group in the sn-3 position, followed by 1,3 lipase acyl ester synthesis. The 2-substituted phospholipid may be used as a functional food ingredient directly, or may be subsequently selectively hydrolyzed in reactor 160 using an immobilized phospholipase C of the invention to produce a 1- diglyceride, followed by enzymatic esterification as described herein to produce a triglyceride product having a 2-substituted polyunsaturated fatty acid component.

The phospholipases and methods of the invention can also be used in a vegetable oil enzymatic degumming process as described, e.g., in U.S. Patent No. 6,001,640. This method of the invention comprises a degumming step in the production of edible oils. Vegetable oils from which hydratable phosphatides have been eliminated by a previous aqueous degumming process are freed from non- hydratable phosphatides by enzymatic treatment using a phospholipase of the invention. The process can be gentle, economical and environment-friendly. Phospholipases that only hydrolyze lysolecithin, but not lecithin, are used in this degumming process.

In one aspect, to allow the enzyme of the invention to act, both phases, the oil phase and the aqueous phase that contain the enzyme, must be intimately mixed. It may not be sufficient to merely stir them. Good dispersion of the enzyme in the oil is aided if it is dissolved in a small amount of water, e.g., 0.5-5 weight-% (relative to the oil), and emulsified in the oil in this form, to form droplets of less than 10 micrometers in diameter (weight average). The droplets can be smaller than 1 micrometer. Turbulent stirring can be done with radial velocities above 100 cm/sec. The oil also can be circulated in the reactor using an external rotary pump. The aqueous phase containing the enzyme can also be finely dispersed by means of ultrasound action. A dispersion apparatus can be used.

The enzymatic reaction probably takes place at the border surface between the oil phase and the aqueous phase. It is the goal of all these measures for mixing to create the

greatest possible surface for the aqueous phase which contains the enzyme. The addition of surfactants increases the microdispersion of the aqueous phase. In some cases, therefore, surfactants with HLB values above 9, such as Na-dodecyl sulfate, are added to the enzyme solution, as described, e.g., in EP-A 0 513 709. A similar effective method for improving emulsification is the addition of lysolecithin. The amounts added can lie in the range of 0.001% to 1%, with reference to the oil. The temperature during enzyme treatment is not critical. Temperatures between 20°C and 80°C can be used, but the latter can only be applied for a short time. In this aspect, a phospholipase of the invention having a good temperature and/or low pH tolerance is used. Application temperatures of between 30°C and 50°C are optimal. The treatment period depends on the temperature and can be kept shorter with an increasing temperature. Times of 0.1 to 10 hours, or, 1 to 5 hours are generally sufficient. The reaction takes place in a degumming reactor, which can be divided into stages, as described, e.g., in DE-A 43 39 556. Therefore continuous operation is possible, along with batch operation. The reaction can be carried out in different temperature stages. For example, incubation can take place for 3 hours at 40°C, then for 1 hour at 60°C. If the reaction proceeds in stages, this also opens up the possibility of adjusting different pH values in the individual stages. For example, in the first stage the pH of the solution can be adjusted to 7, for example, and in a second stage to 2.5, by adding citric acid. In at least one stage, however, the pH of the enzyme solution must be below 4, or, below 3. If the pH was subsequently adjusted below this level, a deterioration of effect may be found. Therefore the citric acid can be added to the enzyme solution before the latter is mixed into the oil.

After completion of the enzyme treatment, the enzyme solution, together with the decomposition products of the NHP contained in it, can be separated from the oil phase, in batches or continuously, e.g., by means of centrifugation. Since the enzymes are characterized by a high level of stability and the amount of the decomposition products contained in the solution is slight (they may precipitate as sludge) the same aqueous enzyme phase can be used several times. There is also the possibility of freeing the enzyme of the sludge, see, e.g., DE-A 43 39 556, so that an enzyme solution which is essentially free of sludge can be used again. In one aspect of this degumming process, oils which contain less than 15 ppm phosphorus are obtained. One goal is phosphorus contents of less than 10 ppm; or, less than 5 ppm. With phosphorus contents below 10 ppm, further processing of the oil according to the process of distillative de-acidification is easily possible. A number of other ions, such as magnesium, calcium, zinc, as well as iron, can be removed from the oil, e.g.,

below 0.1 ppm. Thus, this product possesses ideal prerequisites for good oxidation resistance during further processing and storage.

The phospholipases and methods of the invention also can also be used for reducing the amount of phosphorous-containing components in vegetable and animal oils as described, e.g., in EP patent EP 0513709. In this method, the content of phosphorus-containing components, especially phosphatides, such as lecithin, and the iron content in vegetable and animal oils, which have previously been deslimed, e.g. soya oil, are reduced by enzymatic breakdown using a phospholipase A1, A2 or B of the invention.

The phospholipases and methods of the invention can also be used for refining fat or oils as described, e.g., in JP 06306386. The invention provides processes for refining a fat or oil comprising a step of converting a phospholipid in a fat or an oil into a water-soluble phosphoric-group-containing substance and removing this substance. The action of an enzyme of the invention (e.g., a PLC) is utilized to convert the phospholipid into the substance. Thus, it is possible to refine a fat or oil without carrying out an alkali refining step from which industrial wastes containing alkaline waste water and a large amount of oil are produced. Improvement of yields can be accomplished because the loss of neutral fat or oil from escape with the wastes can be reduced to zero. In one aspect, gummy substances are converted into water-soluble substances and removed as water-soluble substances by adding an enzyme of the invention having a phospholipase C activity in the stage of degumming the crude oil and conducting enzymatic treatment. In one aspect, the phospholipase C of the invention has an activity that cuts ester bonds of glycerin and phosphoric acid in phospholipids. If necessary, the method can comprise washing the enzyme-treated oil with water or an acidic aqueous solution. In one aspect, the enzyme of the invention is added to and reacted with the crude oil. The amount of phospholipase C employed can be 10 to 10,000 units, or, about 100 to 2,000 units, per 1 kg of crude oil.

The phospholipases and methods of the invention can also be used for water-degumming processes as described, e.g., in Dijkstra, Albert J., et al., Oleagineux, Corps Gras, Lipides (1998), 5(5), 367-370. In this exemplary method, the water-degumming process is used for the production of lecithin and for dry degumming processes using a degumming acid and bleaching earth. This method may be economically feasible only for oils with a low phosphatide content, e.g., palm oil, lauric oils, etc. For seed oils having a high NHP-content, the acid refining process is used, whereby this process is carried out at the oil mill to allow gum disposal via the meal. In one aspect, this acid refined oil is a possible "polishing" operation to be carried out prior to physical refining.

The phospholipases and methods of the invention can also be used for degumming processes as described, e.g., in Dijkstra, et al., Res. Dev. Dep., N.V. Vandemoortele Coord. Cent., Izegem, Belg. JAOCS, J. Am. Oil Chem. Soc. (1989), 66:1002-1009. In this exemplary method, the total degumming process involves dispersing an acid such as H_3PO_4 or citric acid into soybean oil, allowing a contact time, and then mixing a base such as caustic soda or Na silicate into the acid-in-oil emulsion. This keeps the degree of neutralization low enough to avoid forming soaps, because that would lead to increased oil loss. Subsequently, the oil passed to a centrifugal separator where most of the gums are removed from the oil stream to yield a gum phase with minimal oil content. The oil stream is then passed to a second centrifugal separator to remove all remaining gums to yield a dilute gum phase, which is recycled. Washing and drying or in-line alkali refining complete the process. After the adoption of the total degumming process, in comparison with the classical alkali refining process, an overall yield improvement of about 0.5% is realized. The totally degummed oil can be subsequently alkali refined, bleached and deodorized, or bleached and physically refined.

The phospholipases and methods of the invention can also be used for the removal of nonhydratable phospholipids from a plant oil, e.g., soybean oil, as described, e.g., in Hvolby, et al., Sojakagefabr., Copenhagen, Den., J. Amer. Oil Chem. Soc. (1971) 48:503-509. In this exemplary method, water-degummed oil is mixed at different fixed pH values with buffer solutions with and without Ca^{++}, Mg/Ca-binding reagents, and surfactants. The nonhydratable phospholipids can be removed in a nonconverted state as a component of micelles or of mixed emulsifiers. Furthermore, the nonhydratable phospholipids are removable by conversion into dissociated forms, e.g., by removal of Mg and Ca from the phosphatidates, which can be accomplished by acidulation or by treatment with Mg/Ca-complexing or Mg/Ca-precipitating reagents. Removal or chemical conversion of the nonhydratable phospholipids can result in reduced emulsion formation and in improved separation of the deacidified oil from the emulsion layer and the soapstock.

The phospholipases and methods of the invention can also be used for the degumming of vegetable oils as described, e.g., Buchold, et al., Frankfurt/Main, Germany. Fett Wissenschaft Technologie (1993), 95(8), 300-304. In this exemplary process of the invention for the degumming of edible vegetable oils, aqueous suspensions of an enzyme of the invention, e.g., phospholipase A2, is used to hydrolyze the fatty acid bound at the sn2 position of the phospholipid, resulting in 1-acyl-lysophospholipids which are insoluble in oil and thus more amenable to physical separation. Even the addition of small amounts

corresponding to about 700 lecitase units/kg oil results in a residual P concentration of less than 10 ppm, so that chemical refining is replaceable by physical refining, eliminating the necessity for neutralization, soapstock splitting, and wastewater treatment.

The phospholipases and methods of the invention can also be used for the degumming of vegetable oils as described, e.g., by EnzyMax. Dahlke, Klaus. Dept. G-PDO, Lurgi Ol-Gas, Chemie, GmbH, Frankfurt, Germany. Oleagineux, Corps Gras, Lipides (1997), 4(1), 55-57. This exemplary process is a degumming process for the physical refining of almost any kind of oil. By an enzymatic-catalyzed hydrolysis, phosphatides are converted to water-soluble lysophosphatides which are separated from the oil by centrifugation. The residual phosphorus content in the enzymatically degummed oil can be as low as 2 ppm P.

The phospholipases and methods of the invention can also be used for the degumming of vegetable oils as described, e.g., by Cleenewerck, et al., N.V. Vamo Mills, Izegem, Belg. Fett Wissenschaft Technologie (1992), 94:317-22; and, Clausen, Kim; Nielsen, Munk. Novozymes A/S, Den. Dansk Kemi (2002) 83(2):24-27. The phospholipases and methods of the invention can incorporate the pre-refining of vegetable oils with acids as described, e.g., by Nilsson-Johansson, et al., Fats Oils Div., Alfa-Laval Food Eng. AB, Tumba, Swed. Fett Wissenschaft Technologie (1988), 90(11), 447-51; and, Munch, Ernst W. Cereol Deutschland GmbH, Mannheim, Germany. Editor(s): Wilson, Richard F. Proceedings of the World Conference on Oilseed Processing Utilization, Cancun, Mexico, Nov. 12-17, 2000 (2001), Meeting Date 2000, 17-20.

The phospholipases and methods of the invention can also be used for the degumming of vegetable oils as described, e.g., by Jerzewska, et al., Inst. Przemyslu Miesnego i Tluszczowego, Warsaw, Pol., Tluszcze Jadalne (2001), 36(3/4), 97-110. In this process of the invention, enzymatic degumming of hydrated low-erucic acid rapeseed oil is by use of a phospholipase A2 of the invention. The enzyme can catalyze the hydrolysis of fatty acid ester linkages to the central carbon atom of the glycerol moiety in phospholipids. It can hydrolyze non-hydratable phospholipids to their corresponding hydratable lyso-compounds. With a nonpurified enzyme preparation, better results can be achieved with the addition of 2% preparation for 4 hours (87% P removal).

Purification of phytosterols from vegetable oils

The invention provides methods for purification of phytosterols and triterpenes, or plant sterols, from vegetable oils. Phytosterols that can be purified using

phospholipases and methods of the invention include β-sitosterol, campesterol, stigmasterol, stigmastanol, β-sitostanol, sitostanol, desmosterol, chalinasterol, poriferasterol, clionasterol and brassicasterol. Plant sterols are important agricultural products for health and nutritional industries. Thus, phospholipases and methods of the invention are used to make emulsifiers for cosmetic manufacturers and steroidal intermediates and precursors for the production of hormone pharmaceuticals. Phospholipases and methods of the invention are used to make (e.g., purify) analogs of phytosterols and their esters for use as cholesterol-lowering agents with cardiologic health benefits. Phospholipases and methods of the invention are used to purify plant sterols to reduce serum cholesterol levels by inhibiting cholesterol absorption in the intestinal lumen. Phospholipases and methods of the invention are used to purify plant sterols that have immunomodulating properties at extremely low concentrations, including enhanced cellular response of T lymphocytes and cytotoxic ability of natural killer cells against a cancer cell line. Phospholipases and methods of the invention are used to purify plant sterols for the treatment of pulmonary tuberculosis, rheumatoid arthritis, management of HIV-infested patients and inhibition of immune stress, e.g., in marathon runners.

Phospholipases and methods of the invention are used to purify sterol components present in the sterol fractions of commodity vegetable oils (e.g., coconut, canola, cocoa butter, corn, cottonseed, linseed, olive, palm, peanut, rice bran, safflower, sesame, soybean, sunflower oils), such as sitosterol (40.2-92.3 %), campesterol (2.6-38.6 %), stigmasterol (0-31 %) and 5-avenasterol (1.5 –29 %).

Methods of the invention can incorporate isolation of plant-derived sterols in oil seeds by solvent extraction with chloroform-methanol, hexane, methylene chloride, or acetone, followed by saponification and chromatographic purification for obtaining enriched total sterols. Alternatively, the plant samples can be extracted by supercritical fluid extraction with supercritical carbon dioxide to obtain total lipid extracts from which sterols can be enriched and isolated. For subsequent characterization and quantification of sterol compounds, the crude isolate can be purified and separated by a wide variety of chromatographic techniques including column chromatography (CC), gas chromatography, thin-layer chromatography (TLC), normal phase high-performance liquid chromatography (HPLC), reversed-phase HPLC and capillary electrochromatography. Of all chromatographic isolation and separation techniques, CC and TLC procedures employ the most accessible, affordable and suitable for sample clean up, purification, qualitative assays and preliminary estimates of the sterols in test samples.

Phytosterols are lost in the vegetable oils lost as byproducts during edible oil refining processes. Phospholipases and methods of the invention use phytosterols isolated from such byproducts to make phytosterol-enriched products isolated from such byproducts. Phytosterol isolation and purification methods of the invention can incorporate oil processing industry byproducts and can comprise operations such as molecular distillation, liquid-liquid extraction and crystallization.

Methods of the invention can incorporate processes for the extraction of lipids to extract phytosterols. For example, methods of the invention can use nonpolar solvents as hexane (commonly used to extract most types of vegetable oils) quantitatively to extract free phytosterols and phytosteryl fatty-acid esters. Steryl glycosides and fatty-acylated steryl glycosides are only partially extracted with hexane, and increasing polarity of the solvent gave higher percentage of extraction. One procedure that can be used is the Bligh and Dyer chloroform-methanol method for extraction of all sterol lipid classes, including phospholipids. One exemplary method to both qualitatively separate and quantitatively analyze phytosterol lipid classes comprises injection of the lipid extract into HPLC system.

Phospholipases and methods of the invention can be used to remove sterols from fats and oils, as described, e.g., in U.S. Patent No. 6,303,803. This is a method for reducing sterol content of sterol-containing fats and oils. It is an efficient and cost effective process based on the affinity of cholesterol and other sterols for amphipathic molecules that form hydrophobic, fluid bilayers, such as phospholipid bilayers. Aggregates of phospholipids are contacted with, for example, a sterol-containing fat or oil in an aqueous environment and then mixed. The molecular structure of this aggregated phospholipid mixture has a high affinity for cholesterol and other sterols, and can selectively remove such molecules from fats and oils. The aqueous separation mixture is mixed for a time sufficient to selectively reduce the sterol content of the fat/oil product through partitioning of the sterol into the portion of phospholipid aggregates. The sterol-reduced fat or oil is separated from the aqueous separation mixture. Alternatively, the correspondingly sterol-enriched fraction also may be isolated from the aqueous separation mixture. These steps can be performed at ambient temperatures, costs involved in heating are minimized, as is the possibility of thermal degradation of the product. Additionally, a minimal amount of equipment is required, and since all required materials are food grade, the methods require no special precautions regarding handling, waste disposal, or contamination of the final product(s).

Phospholipases and methods of the invention can be used to remove sterols from fats and oils, as described, e.g., in U.S. Patent No. 5,880,300. Phospholipid aggregates

are contacted with, for example, a sterol-containing fat or oil in an aqueous environment and then mixed. Following adequate mixing, the sterol-reduced fat or oil is separated from the aqueous separation mixture. Alternatively, the correspondingly sterol-enriched phospholipid also may be isolated from the aqueous separation mixture. Plant (e.g., vegetable) oils contain plant sterols (phytosterols) that also may be removed using the methods of the present invention. This method is applicable to a fat/oil product at any stage of a commercial processing cycle. For example, the process of the invention may be applied to refined, bleached and deodorized oils ("RBD oils"), or to any stage of processing prior to attainment of RBD status. Although RBD oil may have an altered density compared to pre-RBD oil, the processes of the are readily adapted to either RBD or pre-RBD oils, or to various other fat/oil products, by variation of phospholipid content, phospholipid composition, phospholipid:water ratios, temperature, pressure, mixing conditions, and separation conditions as described below.

Alternatively, the enzymes and methods of the invention can be used to isolate phytosterols or other sterols at intermediate steps in oil processing. For example, it is known that phytosterols are lost during deodorization of plant oils. A sterol-containing distillate fraction from, for example, an intermediate stage of processing can be subjected to the sterol-extraction procedures described above. This provides a sterol-enriched lecithin or other phospholipid material that can be further processed in order to recover the extracted sterols.

Detergent Compositions

The invention provides detergent compositions comprising one or more phospholipase of the invention, and methods of making and using these compositions. The invention incorporates all methods of making and using detergent compositions, see, e.g., U.S. Patent No. 6,413,928; 6,399,561; 6,365,561; 6,380,147. The detergent compositions can be a one and two part aqueous composition, a non-aqueous liquid composition, a cast solid, a granular form, a particulate form, a compressed tablet, a gel and/or a paste and a slurry form. The invention also provides methods capable of a rapid removal of gross food soils, films of food residue and other minor food compositions using these detergent compositions. Phospholipases of the invention can facilitate the removal of stains by means of catalytic hydrolysis of phospholipids. Phospholipases of the invention can be used in dishwashing detergents in textile laundering detergents.

The actual active enzyme content depends upon the method of manufacture of a detergent composition and is not critical, assuming the detergent solution has the desired

enzymatic activity. In one aspect, the amount of phospholipase present in the final solution ranges from about 0.001 mg to 0.5 mg per gram of the detergent composition. The particular enzyme chosen for use in the process and products of this invention depends upon the conditions of final utility, including the physical product form, use pH, use temperature, and soil types to be degraded or altered. The enzyme can be chosen to provide optimum activity and stability for any given set of utility conditions. In one aspect, the polypeptides of the present invention are active in the pH ranges of from about 4 to about 12 and in the temperature range of from about 20°C to about 95°C. The detergents of the invention can comprise cationic, semi-polar nonionic or zwitterionic surfactants; or, mixtures thereof.

Phospholipases of the present invention can be formulated into powdered and liquid detergents having pH between 4.0 and 12.0 at levels of about 0.01 to about 5% (preferably 0.1% to 0.5%) by weight. These detergent compositions can also include other enzymes such as known proteases, cellulases, lipases or endoglycosidases, as well as builders and stabilizers. The addition of phospholipases of the invention to conventional cleaning compositions does not create any special use limitation. In other words, any temperature and pH suitable for the detergent is also suitable for the present compositions as long as the pH is within the above range, and the temperature is below the described enzyme's denaturing temperature. In addition, the polypeptides of the invention can be used in a cleaning composition without detergents, again either alone or in combination with builders and stabilizers.

The present invention provides cleaning compositions including detergent compositions for cleaning hard surfaces, detergent compositions for cleaning fabrics, dishwashing compositions, oral cleaning compositions, denture cleaning compositions, and contact lens cleaning solutions.

In one aspect, the invention provides a method for washing an object comprising contacting the object with a phospholipase of the invention under conditions sufficient for washing. A phospholipase of the invention may be included as a detergent additive. The detergent composition of the invention may, for example, be formulated as a hand or machine laundry detergent composition comprising a phospholipase of the invention. A laundry additive suitable for pre-treatment of stained fabrics can comprise a phospholipase of the invention. A fabric softener composition can comprise a phospholipase of the invention. Alternatively, a phospholipase of the invention can be formulated as a detergent composition for use in general household hard surface cleaning operations. In alternative aspects, detergent additives and detergent compositions of the invention may comprise one or

more other enzymes such as a protease, a lipase, a cutinase, another phospholipase, a carbohydrase, a cellulase, a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, an oxidase, e.g., a lactase, and/or a peroxidase. The properties of the enzyme(s) of the invention are chosen to be compatible with the selected detergent (i.e. pH-optimum, compatibility with other enzymatic and non-enzymatic ingredients, etc.) and the enzyme(s) is present in effective amounts. In one aspect, phospholipase enzymes of the invention are used to remove malodorous materials from fabrics. Various detergent compositions and methods for making them that can be used in practicing the invention are described in, e.g., U.S. Patent Nos. 6,333,301; 6,329,333; 6,326,341; 6,297,038; 6,309,871; 6,204,232; 6,197,070; 5,856,164.

Waste treatment

The phospholipases of the invention can be used in waste treatment. In one aspect, the invention provides a solid waste digestion process using phospholipases of the invention. The methods can comprise reducing the mass and volume of substantially untreated solid waste. Solid waste can be treated with an enzymatic digestive process in the presence of an enzymatic solution (including phospholipases of the invention) at a controlled temperature. The solid waste can be converted into a liquefied waste and any residual solid waste. The resulting liquefied waste can be separated from said any residual solidified waste. See e.g., U.S. Patent No. 5,709,796.

Other uses for the phospholipases of the invention

The phospholipases of the invention can also be used to study the phosphoinositide (PI) signaling system; in the diagnosis, prognosis and development of treatments for bipolar disorders (see, e.g., Pandey (2002) Neuropsychopharmacology 26:216-228); as antioxidants; as modified phospholipids; as foaming and gelation agents; to generate angiogenic lipids for vascularizing tissues; to identify phospholipase, e.g, PLA, PLB, PLC, PLD and/or patatin modulators (agonists or antagonists), e.g., inhibitors for use as anti-neoplastics, anti-inflammatory and as analgesic agents. They can be used to generate acidic phospholipids for controlling the bitter taste in food and pharmaceuticals. They can be used in fat purification. They can be used to identify peptides inhibitors for the treatment of viral, inflammatory, allergic and cardiovascular diseases. They can be used to make vaccines. They can be used to make polyunsaturated fatty acid glycerides and phosphatidylglycerols.

The phospholipases of the invention, for example PLA and PLC enzymes, are used to generate immunotoxins and various therapeutics for anti-cancer treatments.

The phospholipases of the invention can be used in conjunction with other enzymes for decoloring (i.e. chlorophyll removal) and in detergents (see above), e.g., in conjunction with other enzymes (e.g., lipases, proteases, esterases, phosphatases). For example, in any instance where a PLC is used, a PLD and a phosphatase may be used in combination, to produce the same result as a PLC alone.

The invention will be further described with reference to the following examples; however, it is to be understood that the invention is not limited to such examples.

EXAMPLES

EXAMPLE 1: BLAST PROGRAM USED FOR SEQUENCE IDENTIFY PROFILNG

This example describes an exemplary sequence identity program to determine if a nucleic acid is within the scope of the invention. An NCBI BLAST 2.2.2 program is used, default options to blastp. All default values were used except for the default filtering setting (i.e., all parameters set to default except filtering which is set to OFF); in its place a "-F F" setting is used, which disables filtering. Use of default filtering often results in Karlin-Altschul violations due to short length of sequence. The default values used in this example:

```
 "Filter for low complexity: ON
> Word Size: 3
> Matrix: Blosum62
> Gap Costs: Existence:11
> Extension:1"
```

Other default settings were: filter for low complexity OFF, word size of 3 for protein, BLOSUM62 matrix, gap existence penalty of -11 and a gap extension penalty of -1. The "-W" option was set to default to 0. This means that, if not set, the word size defaults to 3 for proteins and 11 for nucleotides. The settings read:

```
<<README.bls.txt>>
> ---------------------------------------------------------------------
> blastall arguments:
>
> -p Program Name [String]
> -d Database [String]
>   default = nr
> -i Query File [File In]
>   default = stdin
> -e Expectation value (E) [Real]
>   default = 10.0
> -m alignment view options:
> 0 = pairwise,
```

```
> 1 = query-anchored showing identities,
> 2 = query-anchored no identities,
> 3 = flat query-anchored, show identities,
> 4 = flat query-anchored, no identities,
> 5 = query-anchored no identities and blunt ends,
> 6 = flat query-anchored, no identities and blunt ends,
> 7 = XML Blast output,
> 8 = tabular,
> 9 tabular with comment lines [Integer]
>   default = 0
>  -o  BLAST report Output File [File Out]  Optional
>   default = stdout
>  -F  Filter query sequence (DUST with blastn, SEG with others) [String]
>   default = T
>  -G  Cost to open a gap (zero invokes default behavior) [Integer]
>   default = 0
>  -E  Cost to extend a gap (zero invokes default behavior) [Integer]
>   default = 0
>  -X  X dropoff value for gapped alignment (in bits) (zero invokes default
> behavior) [Integer]
>   default = 0
>  -I  Show GI's in deflines [T/F]
>   default = F
>  -q  Penalty for a nucleotide mismatch (blastn only) [Integer]
>   default = -3
>  -r  Reward for a nucleotide match (blastn only) [Integer]
>   default = 1
>  -v  Number of database sequences to show one-line descriptions for (V)
> [Integer]
>   default = 500
>  -b  Number of database sequence to show alignments for (B) [Integer]
>   default = 250
>  -f  Threshold for extending hits, default if zero [Integer]
>   default = 0
>  -g  Perform gapped alignment (not available with tblastx) [T/F]
>   default = T
>  -Q  Query Genetic code to use [Integer]
>   default = 1
>  -D  DB Genetic code (for tblast[nx] only) [Integer]
>   default = 1
>  -a  Number of processors to use [Integer]
>   default = 1
>  -O  SeqAlign file [File Out]  Optional
>  -J  Believe the query defline [T/F]
>   default = F
>  -M  Matrix [String]
>   default = BLOSUM62
>  -W  Word size, default if zero [Integer]
>   default = 0
>  -z  Effective length of the database (use zero for the real size)
```

```
> [String]
>   default = 0
>  -K  Number of best hits from a region to keep (off by default, if used a
> value of 100 is recommended) [Integer]
>   default = 0
>  -P  0 for multiple hits 1-pass, 1 for single hit 1-pass, 2 for 2-pass
> [Integer]
>   default = 0
>  -Y  Effective length of the search space (use zero for the real size)
> [Real]
>   default = 0
>  -S  Query strands to search against database (for blast[nx], and
> tblastx).  3 is both, 1 is top, 2 is bottom [Integer]
>   default = 3
>  -T  Produce HTML output [T/F]
>   default = F
>  -l  Restrict search of database to list of GI's [String]  Optional
>  -U  Use lower case filtering of FASTA sequence [T/F]  Optional
>   default = F
>  -y  Dropoff (X) for blast extensions in bits (0.0 invokes default
> behavior) [Real]
>   default = 0.0
>  -Z  X dropoff value for final gapped alignment (in bits) [Integer]
>   default = 0
>  -R  PSI-TBLASTN checkpoint file [File In]  Optional
>  -n  MegaBlast search [T/F]
>   default = F
>  -L  Location on query sequence [String]  Optional
>  -A  Multiple Hits window size (zero for single hit algorithm) [Integer]
>   default = 40
```

EXAMPLE 2: SIMULATION OF PLC MEDIATED DEGUMMING

This example describes the simulation of phospholipase C (PLC)-mediated degumming.

Due to its poor solubility in water phosphatidylcholine (PC) was originally dissolved in ethanol (100 mg/ml). For initial testing, a stock solution of PC in 50 mM 3-morpholinopropanesulpholic acid or 60 mM citric acid/NaOH at pH 6 was prepared. The PC stock solution (10µl, 1µg/µl) was added to 500 µl of refined soybean oil (2% water) in an Eppendorf tube. To generate an emulsion the content of the tube was mixed for 3 min by vortexing (see Fig. 5A). The oil and the water phase were separated by centrifugation for 1 min at 13,000 rpm (Fig. 5B). The reaction tubes were pre-incubated at the desired temperature (37^{0}C, 50^{0}C, or 60^{0}C) and 3 µl of PLC from *Bacillus cereus* (0.9 U/µl) were added to the water phase (Fig. 5C). The disappearance of PC was analyzed by TLC using

chloroform/ methanol/water (65:25:4) as a solvent system (see, e.g., Taguchi (1975) supra) and was visualized after exposure to I_2 vapor.

Figure 5 schematically illustrates a model two-phase system for simulation of PLC-mediated degumming. Fig. 5A: Generation of emulsion by mixing crude oil with 2% water to hydrate the contaminating phosphatides (P). Fig. 5B: The oil and water phases are separated after centrifugation and PLC is added to the water phase, which contains the precipitated phosphatides ("gums"). The PLC hydrolysis takes place in the water phase. Fig. 5C: The time course of the reaction is monitored by withdrawing aliquots from the water phase and analyzing them by TLC.

WHAT IS CLAIMED IS:

1. An isolated or recombinant nucleic acid comprising a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, over a region of at least about 100 residues, wherein the nucleic acid encodes at least one polypeptide having a phospholipase activity, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection.

2. The isolated or recombinant nucleic acid of claim 1, wherein the sequence identity is at least about 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63% or 64%.

3. The isolated or recombinant nucleic acid of claim 1, wherein the sequence identity is at least about 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or is 100% sequence identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID

NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105.

4. The isolated or recombinant nucleic acid of claim 1, wherein the sequence identity is over a region of at least about 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or more residues, or the full length of a gene or a transcript.

5. The isolated or recombinant nucleic acid of claim 1, wherein the nucleic acid sequence comprises a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105.

6. The isolated or recombinant nucleic acid of claim 1, wherein the nucleic acid sequence encodes a polypeptide having a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106.

7. The isolated or recombinant nucleic acid of claim 1, wherein the sequence comparison algorithm is a BLAST version 2.2.2 algorithm where a filtering setting is set to blastall -p blastp -d "nr pataa" -F F, and all other options are set to default.

8. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises catalyzing hydrolysis of a glycerolphosphate ester linkage.

9. The isolated or recombinant nucleic acid of claim 8, wherein the phospholipase activity comprises catalyzing hydrolysis of an ester linkage in a phospholipid in a vegetable oil.

10. The isolated or recombinant nucleic acid of claim 8, wherein the vegetable oil phospholipid comprises an oilseed phospholipid.

11. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises a phospholipase C (PLC) activity.

12. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises a phospholipase A (PLA) activity.

13. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises a phospholipase B (PLB) activity.

14. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises a phospholipase D (PLD) activity.

15. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase D activity comprises a phospholipase D1 or a phospholipase D2 activity.

16. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises hydrolysis of a glycoprotein.

17. The isolated or recombinant nucleic acid of claim 16, wherein the glycoprotein comprises a potato tuber.

18. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises a patatin enzymatic activity.

19. The isolated or recombinant nucleic acid of claim 18, wherein the phospholipase activity comprises a lipid acyl hydrolase (LAH) activity.

20. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity is thermostable.

21. The isolated or recombinant nucleic acid of claim 20, wherein the polypeptide retains a phospholipase activity under conditions comprising a temperature range of between about 37°C to about 95°C, or between about 55°C to about 85°C, or between about 70°C to about 75°C, or between about 70°C to about 95°C, or between about 90°C to about 95°C.

22. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity is thermotolerant.

23. The isolated or recombinant nucleic acid of claim 22, wherein the polypeptide retains a phospholipase activity after exposure to a temperature in the range from greater than 37°C to about 95°C, from greater than 55°C to about 85°C, or between about 70°C to about 75°C, or from greater than 90°C to about 95°C.

24. An isolated or recombinant nucleic acid, wherein the nucleic acid comprises a sequence that hybridizes under stringent conditions to a nucleic acid comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID

NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, wherein the nucleic acid encodes a polypeptide having a phospholipase activity.

25. The isolated or recombinant nucleic acid of claim 24, wherein the nucleic acid is at least about 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more residues in length or the full length of the gene or transcript.

26. The isolated or recombinant nucleic acid of claim 24, wherein the stringent conditions include a wash step comprising a wash in 0.2X SSC at a temperature of about 65°C for about 15 minutes.

27. A nucleic acid probe for identifying a nucleic acid encoding a polypeptide with a phospholipase activity, wherein the probe comprises at least 10 consecutive bases of a sequence comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, wherein the probe identifies the nucleic acid by binding or hybridization.

28. The nucleic acid probe of claim 27, wherein the probe comprises an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, about 60 to 100, or about 50 to 150 consecutive bases.

29. A nucleic acid probe for identifying a nucleic acid encoding a polypeptide having a phospholipase activity, wherein the probe comprises a nucleic acid comprising at least about 10 consecutive residues of SEQ ID NO:1; SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ

ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection.

30. The nucleic acid probe of claim 29, wherein the probe comprises an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, about 60 to 100, or about 50 to 150 consecutive bases.

31. An amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having a phospholipase activity, wherein the primer pair is capable of amplifying a nucleic acid comprising a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

32. The amplification primer pair of claim 29, wherein a member of the amplification primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 consecutive bases, or about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 consecutive bases of the sequence.

33. An amplification primer pair, wherein the primer pair comprises a first member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more residues of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69,

SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, and a second member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 or more residues of the complementary strand of the first member.

34. A phospholipase-encoding nucleic acid generated by amplification of a polynucleotide using an amplification primer pair as set forth in claim 33.

35. The phospholipase-encoding nucleic acid of claim 34, wherein the amplification is by polymerase chain reaction (PCR).

36. The phospholipase-encoding nucleic acid of claim 34, wherein the nucleic acid generated by amplification of a gene library.

37. The phospholipase-encoding nucleic acid of claim 34, wherein the gene library is an environmental library.

38. An isolated or recombinant phospholipase encoded by a phospholipase-encoding nucleic acid as set forth in claim 34.

39. A method of amplifying a nucleic acid encoding a polypeptide having a phospholipase activity comprising amplification of a template nucleic acid with an amplification primer sequence pair capable of amplifying a nucleic acid sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

40. A method for making a phospholipase comprising amplification of a nucleic acid with an amplification primer pair as set forth in claim 33 and expression of the amplified nucleic acid.

41. An expression cassette comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

42. A vector comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

43. A cloning vehicle comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24, wherein the cloning vehicle comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome.

44. The cloning vehicle of claim 43, wherein the viral vector comprises an adenovirus vector, a retroviral vector or an adeno-associated viral vector.

45. The cloning vehicle of claim 43, comprising a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC).

46. A transformed cell comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

47. A transformed cell comprising an expression cassette as set forth in claim 41.

48. The transformed cell of claim 47, wherein the cell is a bacterial cell, a mammalian cell, a fungal cell, a yeast cell, an insect cell or a plant cell.

49. A transgenic non-human animal comprising a sequence as set forth in claim 1 or claim 24.

50. The transgenic non-human animal of claim 49, wherein the animal is a mouse.

51. A transgenic plant comprising a sequence as set forth in claim 1 or claim 24.

52. The transgenic plant of claim 51, wherein the plant is a corn plant, a sorghum plant, a potato plant, a tomato plant, a wheat plant, an oilseed plant, a rapeseed plant, a soybean plant, a rice plant, a barley plant, a grass, a cottonseed, a palm, a sesame plant, a peanut plant, a sunflower plant or a tobacco plant.

53. A transgenic seed comprising a sequence as set forth in claim 1 or claim 24.

54. The transgenic seed of claim 53, wherein the seed is a corn seed, a wheat kernel, an oilseed, a rapeseed, a soybean seed, a palm kernel, a sunflower seed, a sesame seed, a rice, a barley, a peanut, a cottonseed, a palm, a peanut, a sesame seed, a sunflower seed or a tobacco plant seed.

55. An antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

56. The antisense oligonucleotide of claim 55, wherein the antisense oligonucleotide is between about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 bases in length.

57. A method of inhibiting the translation of a phospholipase message in a cell comprising administering to the cell or expressing in the cell an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or claim 24.

58. A double-stranded inhibitory RNA (RNAi) molecule comprising a subsequence of a sequence as set forth in claim 1 or claim 24.

59. The double-stranded inhibitory RNA (RNAi) molecule of claim 58, wherein the RNAi is about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more duplex nucleotides in length.

60. A method of inhibiting the expression of a phospholipase in a cell comprising administering to the cell or expressing in the cell a double-stranded inhibitory RNA (iRNA), wherein the RNA comprises a subsequence of a sequence as set forth in claim 1 or claim 24.

61. An isolated or recombinant polypeptide (i) having at least 50% sequence identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, over a region of at least about 100 residues, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, or, (ii) encoded by a nucleic acid having at least 50% sequence identity to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105 over a region of at least about 100 residues, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, or encoded by a nucleic acid capable of hybridizing under stringent conditions to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29,

SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105.

62. The isolated or recombinant polypeptide of claim 61, wherein the sequence identity is over a region of at least about at least about 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or is 100% sequence identity.

63. The isolated or recombinant polypeptide of claim 61, wherein the sequence identity is over a region of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050 or more residues, or the full length of an enzyme.

64. The isolated or recombinant polypeptide of claim 61, wherein the polypeptide has a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106.

65. The isolated or recombinant polypeptide of claim 61, wherein the polypeptide has a phospholipase activity.

66. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises catalyzing hydrolysis of a glycerolphosphate ester linkage.

67. The isolated or recombinant polypeptide of claim 66, wherein the phospholipase activity comprises catalyzing hydrolysis of an ester linkage in a phospholipid in a vegetable oil.

68. The isolated or recombinant polypeptide of claim 67, wherein the vegetable oil phospholipid comprises an oilseed phospholipid.

69. The isolated or recombinant polypeptide of claim 67, wherein the vegetable oil phospholipid is derived from a plant oil, a high phosphorous oil, a soy oil, a canola oil, a palm oil, a cottonseed oil, a corn oil, a palm kernel-derived phospholipid, a coconut oil, a peanut oil, a sesame oil, a fish oil, an algae phospholipid, a sunflower oil, an essential oil, a fruit seed oil, a grapeseed phospholipid, an apricot phospholipid, or a borage phospholipid.

70. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a phospholipase C (PLC) activity.

71. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a phospholipase A (PLA) activity.

72. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase a activity comprises a phospholipase A1 or phospholipase A2 activity.

73. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a phospholipase D (PLD) activity.

74. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase D activity comprises a phospholipase D1 or a phospholipase D2 activity.

75. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises hydrolysis of a glycoprotein.

76. The isolated or recombinant polypeptide of claim 68, wherein the glycoprotein comprises a potato tuber.

77. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a patatin enzymatic activity.

78. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a lipid acyl hydrolase (LAH) activity.

79. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity is thermostable.

80. The isolated or recombinant polypeptide of claim 79, wherein the polypeptide retains a phospholipase activity under conditions comprising a temperature range of between about 37°C to about 95°C, between about 55°C to about 85°C, between about 70°C to about 95°C, between about 70°C to about 75°C, or between about 90°C to about 95°C.

81. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity is thermotolerant.

82. The isolated or recombinant polypeptide of claim 81, wherein the polypeptide retains a phospholipase activity after exposure to a temperature in the range from greater than 37°C to about 95°C, from greater than 55°C to about 85°C, between about 70°C to about 75°C, or from greater than 90°C to about 95°C.

83. An isolated or recombinant polypeptide comprising a polypeptide as set forth in claim 61 and lacking a signal sequence.

84. An isolated or recombinant polypeptide comprising a polypeptide as set forth in claim 61 and having a heterologous signal sequence.

85. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a specific activity at about 37°C in the range from about 100 to about 1000 units per milligram of protein, from about 500 to about 750 units per milligram of protein, from about 500 to about 1200 units per milligram of protein, or from about 750 to about 1000 units per milligram of protein.

86. The isolated or recombinant polypeptide of claim 81, wherein the thermotolerance comprises retention of at least half of the specific activity of the phospholipase at 37°C after being heated to an elevated temperature.

87. The isolated or recombinant polypeptide of claim 81, wherein the thermotolerance comprises retention of specific activity at 37°C in the range from about 500 to about 1200 units per milligram of protein after being heated to an elevated temperature.

88. The isolated or recombinant polypeptide of claim 61, wherein the polypeptide comprises at least one glycosylation site.

89. The isolated or recombinant polypeptide of claim 88, wherein the glycosylation is an N-linked glycosylation.

90. The isolated or recombinant polypeptide of claim 89, wherein the polypeptide is glycosylated after being expressed in an *P. pastoris* or an *S. pombe.*

91. The isolated or recombinant polypeptide of claim 65, wherein the polypeptide retains a phospholipase activity under conditions comprising about pH 6.5, pH 6.0, pH 5.5, 5.0, pH 4.5 or 4.0.

92. The isolated or recombinant polypeptide of claim 65, wherein the polypeptide retains a phospholipase activity under conditions comprising about pH 7.5, pH 8.0, pH 8.5, pH 9, pH 9.5, pH 10 or pH 10.5.

93. A protein preparation comprising a polypeptide as set forth in claim 61, wherein the protein preparation comprises a liquid, a solid or a gel.

94. A heterodimer comprising a polypeptide as set forth in claim 61 and a second domain.

95. The heterodimer of claim 94, wherein the second domain is a polypeptide and the heterodimer is a fusion protein.

96. The heterodimer of claim 94, wherein the second domain is an epitope or a tag.

97. A homodimer comprising a polypeptide as set forth in claim 61.

98. An immobilized polypeptide, wherein the polypeptide comprises a sequence as set forth in claim 61, or a subsequence thereof.

99. The immobilized polypeptide of claim 98, wherein the polypeptide is immobilized on a cell, a metal, a resin, a polymer, a ceramic, a glass, a microelectrode, a graphitic particle, a bead, a gel, a plate, an array or a capillary tube.

100. An array comprising an immobilized polypeptide as set forth in claim 61.

101. An array comprising an immobilized nucleic acid as set forth in claim 1 or claim 24.

102. An isolated or recombinant antibody that specifically binds to a polypeptide as set forth in claim 61.

103. The isolated or recombinant antibody of claim 102, wherein the antibody is a monoclonal or a polyclonal antibody.

104. A hybridoma comprising an antibody that specifically binds to a polypeptide as set forth in claim 61.

105. A method of isolating or identifying a polypeptide with a phospholipase activity comprising the steps of:

(a) providing an antibody as set forth in claim 102;

(b) providing a sample comprising polypeptides; and

(c) contacting the sample of step (b) with the antibody of step (a) under conditions wherein the antibody can specifically bind to the polypeptide, thereby isolating or identifying a polypeptide having a phospholipase activity.

106. A method of making an anti-phospholipase antibody comprising administering to a non-human animal a nucleic acid as set forth in claim 1 or claim 24 or a subsequence thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-phospholipase antibody.

107. A method of making an anti-phospholipase antibody comprising administering to a non-human animal a polypeptide as set forth in claim 61 or a subsequence thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-phospholipase antibody.

108. A method of producing a recombinant polypeptide comprising the steps of: (a) providing a nucleic acid operably linked to a promoter, wherein the nucleic acid comprises a sequence as set forth in claim 1 or claim 24; and (b) expressing the nucleic acid of step (a) under conditions that allow expression of the polypeptide, thereby producing a recombinant polypeptide.

109. The method of claim 108, further comprising transforming a host cell with the nucleic acid of step (a) followed by expressing the nucleic acid of step (a), thereby producing a recombinant polypeptide in a transformed cell.

110. A method for identifying a polypeptide having a phospholipase activity comprising the following steps:

(a) providing a polypeptide as set forth in claim 65;

(b) providing a phospholipase substrate; and

(c) contacting the polypeptide with the substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of a reaction product,

wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product detects a polypeptide having a phospholipase activity.

111. A method for identifying a phospholipase substrate comprising the following steps:

(a) providing a polypeptide as set forth in claim 65;

(b) providing a test substrate; and

(c) contacting the polypeptide of step (a) with the test substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of a reaction product identifies the test substrate as a phospholipase substrate.

112. A method of determining whether a test compound specifically binds to a polypeptide comprising the following steps:

(a) expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide, wherein the nucleic acid has a sequence as set forth in claim 1 or claim 24;

(b) providing a test compound;

(c) contacting the polypeptide with the test compound; and

(d) determining whether the test compound of step (b) specifically binds to the polypeptide.

113. A method of determining whether a test compound specifically binds to a polypeptide comprising the following steps:

(a) providing a polypeptide as set forth in claim 61;

(b) providing a test compound;

(c) contacting the polypeptide with the test compound; and

(d) determining whether the test compound of step (b) specifically binds to the polypeptide.

114. A method for identifying a modulator of a phospholipase activity comprising the following steps:

(a) providing a polypeptide as set forth in claim 65;

(b) providing a test compound;

(c) contacting the polypeptide of step (a) with the test compound of step (b) and measuring an activity of the phospholipase, wherein a change in the phospholipase activity measured in the presence of the test compound compared to the activity in the absence of the test compound provides a determination that the test compound modulates the phospholipase activity.

115. The method of claim 114, wherein the phospholipase activity is measured by providing a phospholipase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product, or, an increase in the amount of the substrate or a decrease in the amount of a reaction product.

116. The method of claim 115, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an activator of phospholipase activity.

117. The method of claim 115, wherein an increase in the amount of the substrate or a decrease in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an inhibitor of phospholipase activity.

118. A computer system comprising a processor and a data storage device wherein said data storage device has stored thereon a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises sequence as set forth in claim 61, a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

119. The computer system of claim 118, further comprising a sequence comparison algorithm and a data storage device having at least one reference sequence stored thereon.

120. The computer system of claim 119, wherein the sequence comparison algorithm comprises a computer program that indicates polymorphisms.

121. The computer system of claim 119, further comprising an identifier that identifies one or more features in said sequence.

122. A computer readable medium having stored thereon a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 61; a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

123. A method for identifying a feature in a sequence comprising the steps of: (a) reading the sequence using a computer program which identifies one or more features in a sequence, wherein the sequence comprises a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 61; a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and (b) identifying one or more features in the sequence with the computer program.

124. A method for comparing a first sequence to a second sequence comprising the steps of: (a) reading the first sequence and the second sequence through use of a computer program which compares sequences, wherein the first sequence comprises a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 61 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and (b) determining differences between the first sequence and the second sequence with the computer program.

125. The method of claim 124, wherein the step of determining differences between the first sequence and the second sequence further comprises the step of identifying polymorphisms.

126. The method of claim 124, further comprising an identifier that identifies one or more features in a sequence.

127. The method of claim 126, comprising reading the first sequence using a computer program and identifying one or more features in the sequence.

128. A method for isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample comprising the steps of:

(a) providing an amplification primer sequence pair as set forth in claim 33;

(b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair; and,

(c) combining the nucleic acid of step (b) with the amplification primer pair of step (a) and amplifying nucleic acid from the environmental sample, thereby isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample.

129. The method of claim 128, wherein each member of the amplification primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 consecutive bases of a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, or a subsequence thereof.

130. A method for isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample comprising the steps of:

(a) providing a polynucleotide probe comprising a sequence as set forth in claim 1 or claim 24, or a subsequence thereof;

(b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe of step (a);

(c) combining the isolated nucleic acid or the treated environmental sample of step (b) with the polynucleotide probe of step (a); and

(d) isolating a nucleic acid that specifically hybridizes with the polynucleotide probe of step (a), thereby isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample.

131. The method of claim 128 or claim 130, wherein the environmental sample comprises a water sample, a liquid sample, a soil sample, an air sample or a biological sample.

132. The method of claim 131, wherein the biological sample is derived from a bacterial cell, a protozoan cell, an insect cell, a yeast cell, a plant cell, a fungal cell or a mammalian cell.

133. A method of generating a variant of a nucleic acid encoding a polypeptide with a phospholipase activity comprising the steps of:

(a) providing a template nucleic acid comprising a sequence as set forth in claim 1 or claim 24; and

(b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid.

134. The method of claim 133, further comprising expressing the variant nucleic acid to generate a variant phospholipase polypeptide.

135. The method of claim 133, wherein the modifications, additions or deletions are introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSM), synthetic ligation reassembly (SLR) and a combination thereof.

136. The method of claim 133, wherein the modifications, additions or deletions are introduced by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template

mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

137. The method of claim 133, wherein the method is iteratively repeated until a phospholipase having an altered or different activity or an altered or different stability from that of a polypeptide encoded by the template nucleic acid is produced.

138. The method of claim 137, wherein the variant phospholipase polypeptide is thermotolerant, and retains some activity after being exposed to an elevated temperature.

139. The method of claim 137, wherein the variant phospholipase polypeptide has increased glycosylation as compared to the phospholipase encoded by a template nucleic acid.

140. The method of claim 137, wherein the variant phospholipase polypeptide has a phospholipase activity under a high temperature, wherein the phospholipase encoded by the template nucleic acid is not active under the high temperature.

141. The method of claim 133, wherein the method is iteratively repeated until a phospholipase coding sequence having an altered codon usage from that of the template nucleic acid is produced.

142. The method of claim 133, wherein the method is iteratively repeated until a phospholipase gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced.

143. A method for modifying codons in a nucleic acid encoding a polypeptide with a phospholipase activity to increase its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding a polypeptide with a phospholipase activity comprising a sequence as set forth in claim 1 or claim 24; and,

(b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

144. A method for modifying codons in a nucleic acid encoding a phospholipase polypeptide, the method comprising the following steps:

(a) providing a nucleic acid encoding a polypeptide with a phospholipase activity comprising a sequence as set forth in claim 1 or claim 24; and,

(b) identifying a codon in the nucleic acid of step (a) and replacing it with a different codon encoding the same amino acid as the replaced codon, thereby modifying codons in a nucleic acid encoding a phospholipase.

145. A method for modifying codons in a nucleic acid encoding a phospholipase polypeptide to increase its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding a phospholipase polypeptide comprising a sequence as set forth in claim 1 or claim 24; and,

(b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

146. A method for modifying a codon in a nucleic acid encoding a polypeptide having a phospholipase activity to decrease its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding a phospholipase polypeptide comprising a sequence as set forth in claim 1 or claim 24; and

(b) identifying at least one preferred codon in the nucleic acid of step (a) and replacing it with a non-preferred or less preferred codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in a host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to decrease its expression in a host cell.

147. The method of claim 146, wherein the host cell is a bacterial cell, a fungal cell, an insect cell, a yeast cell, a plant cell or a mammalian cell.

148. A method for producing a library of nucleic acids encoding a plurality of modified phospholipase active sites or substrate binding sites, wherein the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site the method comprising the following steps:

(a) providing a first nucleic acid encoding a first active site or first substrate binding site, wherein the first nucleic acid sequence comprises a sequence that hybridizes under stringent conditions to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, or a subsequence thereof, and the nucleic acid encodes a phospholipase active site or a phospholipase substrate binding site;

(b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

(c) using the set of mutagenic oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library

of nucleic acids encoding a plurality of modified phospholipase active sites or substrate binding sites.

149. The method of claim 148, comprising mutagenizing the first nucleic acid of step (a) by a method comprising an optimized directed evolution system, gene site-saturation mutagenesis (GSSM), or a synthetic ligation reassembly (SLR).

150. The method of claim 148, comprising mutagenizing the first nucleic acid of step (a) or variants by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSM), synthetic ligation reassembly (SLR) and a combination thereof.

151. The method of claim 148, comprising mutagenizing the first nucleic acid of step (a) or variants by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

152. A method for making a small molecule comprising the following steps:

(a) providing a plurality of biosynthetic enzymes capable of synthesizing or modifying a small molecule, wherein one of the enzymes comprises a phospholipase enzyme encoded by a nucleic acid comprising a sequence as set forth in claim 1 or claim 24;

(b) providing a substrate for at least one of the enzymes of step (a); and

(c) reacting the substrate of step (b) with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series of biocatalytic reactions.

153. A method for modifying a small molecule comprising the following steps:

(a) providing a phospholipase enzyme, wherein the enzyme comprises a polypeptide as set forth in claim 65, or a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence as set forth in claim 1 or claim 24;

(b) providing a small molecule; and

(c) reacting the enzyme of step (a) with the small molecule of step (b) under conditions that facilitate an enzymatic reaction catalyzed by the phospholipase enzyme, thereby modifying a small molecule by a phospholipase enzymatic reaction.

154. The method of claim 153, comprising a plurality of small molecule substrates for the enzyme of step (a), thereby generating a library of modified small molecules produced by at least one enzymatic reaction catalyzed by the phospholipase enzyme.

155. The method of claim 153, further comprising a plurality of additional enzymes under conditions that facilitate a plurality of biocatalytic reactions by the enzymes to form a library of modified small molecules produced by the plurality of enzymatic reactions.

156. The method of claim 155, further comprising the step of testing the library to determine if a particular modified small molecule which exhibits a desired activity is present within the library.

157. The method of claim 156, wherein the step of testing the library further comprises the steps of systematically eliminating all but one of the biocatalytic reactions used to produce a portion of the plurality of the modified small molecules within the library by testing the portion of the modified small molecule for the presence or absence of the particular modified small molecule with a desired activity, and identifying at least one specific biocatalytic reaction that produces the particular modified small molecule of desired activity.

158. A method for determining a functional fragment of a phospholipase enzyme comprising the steps of:

(a) providing a phospholipase enzyme, wherein the enzyme comprises a polypeptide as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and

(b) deleting a plurality of amino acid residues from the sequence of step (a) and testing the remaining subsequence for a phospholipase activity, thereby determining a functional fragment of a phospholipase enzyme.

159. The method of claim 158, wherein the phospholipase activity is measured by providing a phospholipase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product.

160. A method for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis, the method comprising the following steps:

(a) making a modified cell by modifying the genetic composition of a cell, wherein the genetic composition is modified by addition to the cell of a nucleic acid comprising a sequence as set forth in claim 1 or claim 24;

(b) culturing the modified cell to generate a plurality of modified cells;

(c) measuring at least one metabolic parameter of the cell by monitoring the cell culture of step (b) in real time; and,

(d) analyzing the data of step (c) to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions, thereby identifying an engineered phenotype in the cell using real-time metabolic flux analysis.

161. The method of claim 160, wherein the genetic composition of the cell is modified by a method comprising deletion of a sequence or modification of a sequence in the cell, or, knocking out the expression of a gene.

162. The method of claim 160, further comprising selecting a cell comprising a newly engineered phenotype.

163. The method of claim 162, further comprising culturing the selected cell, thereby generating a new cell strain comprising a newly engineered phenotype.

164. An isolated or recombinant signal sequence consisting of a sequence as set forth in residues 1 to 16, 1 to 17, 1 to 18, 1 to 19, 1 to 20, 1 to 21, 1 to 22, 1 to 23, 1 to 24, 1 to 25, 1 to 26, 1 to 27, 1 to 28, 1 to 28, 1 to 30 or 1 to 31, 1 to 32 or 1 to 33 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106.

165. A chimeric polypeptide comprising at least a first domain comprising signal peptide (SP) having a sequence as set forth in claim 164, and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP).

166. The chimeric polypeptide of claim 165, wherein the heterologous polypeptide or peptide is not a phospholipase.

167. The chimeric polypeptide of claim 165, wherein the heterologous polypeptide or peptide is amino terminal to, carboxy terminal to or on both ends of the signal peptide (SP) or a catalytic domain (CD).

168. An isolated or recombinant nucleic acid encoding a chimeric polypeptide, wherein the chimeric polypeptide comprises at least a first domain comprising signal peptide (SP having a sequence as set forth in claim 164 and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP).

169. A method of increasing thermotolerance or thermostability of a phospholipase polypeptide, the method comprising glycosylating a phospholipase, wherein

the polypeptide comprises at least thirty contiguous amino acids of a polypeptide as set forth in claim 61, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, thereby increasing the thermotolerance or thermostability of the phospholipase.

170. A method for overexpressing a recombinant phospholipase in a cell comprising expressing a vector comprising a nucleic acid sequence as set forth in claim 1 or claim 24, wherein overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector.

171. A method of making a transgenic plant comprising the following steps:

(a) introducing a heterologous nucleic acid sequence into the cell, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 or claim 24, thereby producing a transformed plant cell;

(b) producing a transgenic plant from the transformed cell.

172. The method as set forth in claim 171, wherein the step (a) further comprises introducing the heterologous nucleic acid sequence by electroporation or microinjection of plant cell protoplasts.

173. The method as set forth in claim 171, wherein the step (a) comprises introducing the heterologous nucleic acid sequence directly to plant tissue by DNA particle bombardment or by using an Agrobacterium tumefaciens host.

174. A method of expressing a heterologous nucleic acid sequence in a plant cell comprising the following steps:

(a) transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 or claim 24;

(b) growing the plant under conditions wherein the heterologous nucleic acids sequence is expressed in the plant cell.

175. A method for hydrolyzing, breaking up or disrupting a phospholipid-comprising composition comprising the following steps:

(a) providing a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing a composition comprising a phospholipid; and

(c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the phospholipase hydrolyzes, breaks up or disrupts the phospholipid-comprising composition.

176. The method as set forth in claim 175, wherein the composition comprises a phospholipid-comprising lipid bilayer or membrane.

177. The method as set forth in claim 175, wherein the composition comprises a plant cell, a bacterial cell, a yeast cell, an insect cell, or an animal cell.

178. A method for liquefying or removing a phospholipid-comprising composition comprising the following steps:

(a) providing a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing a composition comprising a phospholipid; and

(c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the phospholipase removes or liquefies the phospholipid-comprising composition.

179. A detergent composition comprising a polypeptide as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, wherein the polypeptide has a phospholipase activity.

180. The detergent composition of claim 179, wherein the phospholipase is a nonsurface-active phospholipase or a surface-active phospholipase.

181. The detergent composition of claim 179, wherein the phospholipase is formulated in a non-aqueous liquid composition, a cast solid, a granular form, a particulate form, a compressed tablet, a gel form, a paste or a slurry form.

182. A method for washing an object comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing an object; and

(c) contacting the polypeptide of step (a) and the object of step (b) under conditions wherein the composition can wash the object.

183. A method for degumming an oil comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing an composition comprising an phospholipid-containing fat or oil; and

(c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

184. The method of claim 183, wherein the oil-comprising composition comprises a plant, an animal, an algae or a fish oil or fat.

185. The method of claim 184, wherein plant oil comprises a soybean oil, a rapeseed oil, a corn oil, an oil from a palm kernel, a canola oil, a sunflower oil, a sesame oil or a peanut oil.

186. The method of claim 183, wherein the polypeptide hydrolyzes a phosphatide from a hydratable and/or a non-hydratable phospholipid in the oil-comprising composition.

187. The method of claim 183, wherein the polypeptide hydrolyzes a phosphatide at a glyceryl phosphoester bond to generate a diglyceride and water-soluble phosphate compound.

188. The method of claim 183, wherein the polypeptide has a phospholipase C activity.

189. The method of claim 183, wherein the polypeptide has a phospholipase D activity and a phosphatase enzyme is also added.

190. The method of claim 183, wherein the contacting comprises hydrolysis of a hydrated phospholipid in an oil.

191. The method of claim 183, wherein the hydrolysis conditions of step (c) comprise a temperature of about 20°C to 40°C at an alkaline pH.

192. The method of claim 190, wherein the alkaline conditions comprise a pH of about pH 8 to pH 10.

193. The method of claim 183, wherein the hydrolysis conditions of step (c) comprise a reaction time of about 3 to 10 minutes.

194. The method of claim 183, wherein the hydrolysis conditions of step (c) comprise hydrolysis of hydratable and non-hydratable phospholipids in oil at a temperature of about 50°C to 60°C, at a pH of about pH 5 to pH 6.5 using a reaction time of about 30 to 60 minutes.

195. The method of claim 183, wherein the polypeptide is bound to a filter and the phospholipid-containing fat or oil is passed through the filter.

196. The method of claim 183, wherein the polypeptide is added to a solution comprising the phospholipid-containing fat or oil and then the solution is passed through a filter.

197. A method for converting a non-hydratable phospholipid to a hydratable form comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing an composition comprising a non-hydratable phospholipid; and

(c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the polypeptide converts the non-hydratable phospholipid to a hydratable form.

198. The method of claim 197, wherein the polypeptide has a phospholipase C activity.

199. The method of claim 197, wherein the polypeptide has a phospholipase D activity and a phosphatase enzyme is also added.

200. A method for caustic refining of a phospholipid-containing composition comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing an composition comprising a phospholipid; and

(c) contacting the polypeptide of step (a) with the composition of step (b) before, during or after the caustic refining.

201. The method of claim 200, wherein the polypeptide has a phospholipase C activity.

202. The method of claim 200, wherein the polypeptide having a phospholipase activity is added before caustic refining and the composition comprising the phospholipid comprises a plant and the polypeptide is expressed transgenically in the plant, the polypeptide having a phospholipase activity added during crushing of a seed or other plant part, or, the polypeptide having a phospholipase activity added following crushing or prior to refining.

203. The method of claim 200, wherein the polypeptide having a phospholipase activity is added during caustic refining and varying levels of acid and caustic are added depending on levels of phosphorous and levels of free fatty acids.

204. The method of claim 200, wherein the polypeptide having a phospholipase activity is added after caustic refining: in an intense mixer or retention mixer prior to separation; following a heating step; in a centrifuge; in a soapstock; in a washwater; or, during bleaching or deodorizing steps.

205. A method for purification of a phytosterol or a triterpene comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing an composition comprising a phytosterol or a triterpene; and

(c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

206. The method of claim 205, wherein the polypeptide has a phospholipase C activity.

207. The method of claim 205, wherein the phytosterol or a triterpene comprises a plant sterol.

208. The method of claim 207, wherein the plant sterol is derived from a vegetable oil.

209. The method of claim 208, wherein the vegetable oil comprises a coconut oil, canola oil, cocoa butter oil, corn oil, cottonseed oil, linseed oil, olive oil, palm oil, peanut oil, oil derived from a rice bran, safflower oil, sesame oil, soybean oil or a sunflower oil.

210. The method of claim 205, further comprising use of nonpolar solvents to quantitatively extract free phytosterols and phytosteryl fatty-acid esters.

211. The method of claim 205, wherein the phytosterol or a triterpene comprises a β-sitosterol, a campesterol, a stigmasterol, a stigmastanol, a β-sitostanol, a sitostanol, a desmosterol, a chalinasterol, a poriferasterol, a clionasterol or a brassicasterol.

212. A method for refining a crude oil comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing a composition comprising an oil comprising a phospholipid; and

(c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

213. The method of claim 212, wherein the polypeptide has a phospholipase C activity.

214. The method of claim 212, wherein the polypeptide having a phospholipase activity is in a water solution that is added to the composition.

215. The method of claim 214, wherein the water level is between about 0.5 to 5%.

216. The method of claim 214, wherein the process time is less than about 2 hours.

217. The method of claim 216, wherein the process time is less than about 60 minutes.

218. The method of claim 217, wherein the process time is less than about 30 minutes, less than about 15 minutes, or less than about 5 minutes.

219. The method of claim 212, wherein the hydrolysis conditions comprise a temperature of between about 25°C-70°C.

220. The method of claim 212, wherein the hydrolysis conditions comprise use of caustics.

221. The method of claim 212, wherein the hydrolysis conditions comprise a pH of between about pH 3 and pH 10.

222. The method of claim 212, wherein the hydrolysis conditions comprise addition of emulsifiers and/or mixing after the contacting of step (c).

223. The method of claim 212, comprising addition of an emulsion-breaker and/or heat to promote separation of an aqueous phase.

224. The method of claim 212, comprising degumming before the contacting step to collect lecithin by centrifugation and then adding a PLC, a PLC and/or a PLA to remove non-hydratable phospholipids.

225. The method of claim 212, comprising water degumming of crude oil to less than 10 ppm for edible oils and subsequent physical refining to less than about 50 ppm for biodiesel oils.

226. The method of claim 212, comprising addition of acid to promote hydration of non-hydratable phospholipids.

227. A method for degumming an oil or a fat comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, wherein the phospholipase activity comprises a phospholipase D activity, and a phosphatase enzyme;

(b) providing an composition comprising an phospholipid-containing fat or oil; and

(c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

228. A composition having the equivalent of a phospholipase C activity comprising providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, wherein the phospholipase activity comprises a phospholipase D activity, and a phosphatase enzyme.

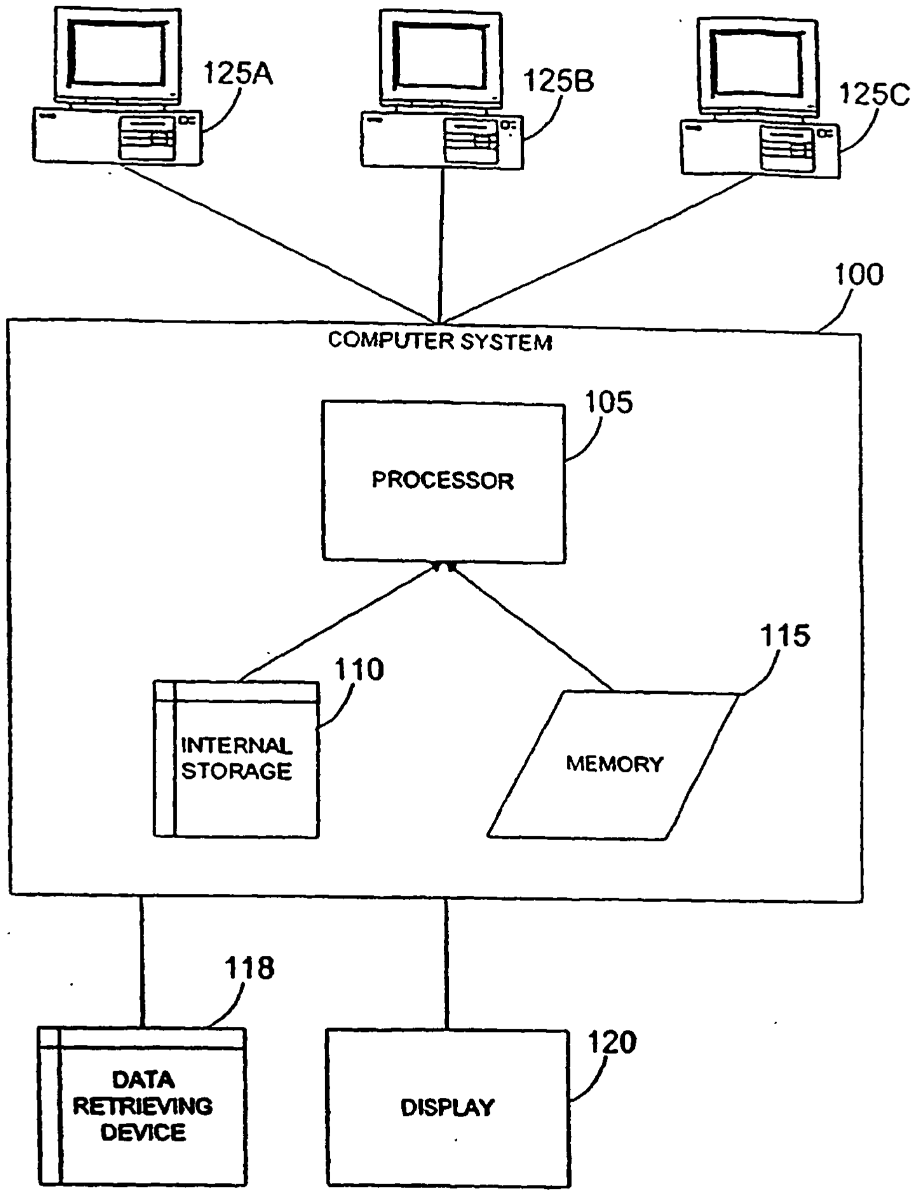

125A
125B
125C
100
COMPUTER SYSTEM
105
PROCESSOR
110
INTERNAL STORAGE
115
MEMORY
118
DATA RETRIEVING DEVICE
120
DISPLAY

FIGURE 1

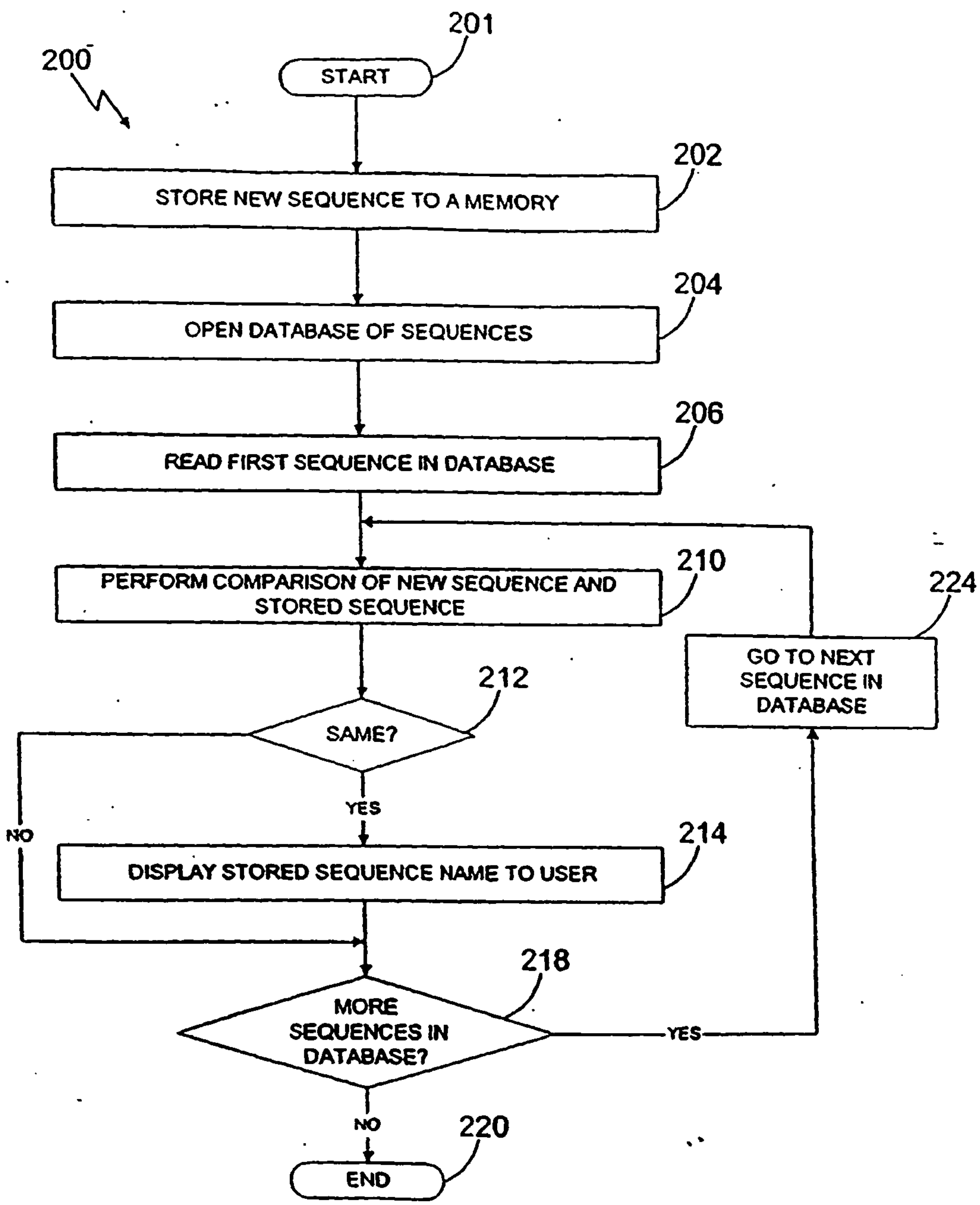

200
201
START
202
STORE NEW SEQUENCE TO A MEMORY
204
OPEN DATABASE OF SEQUENCES
206
READ FIRST SEQUENCE IN DATABASE
210
PERFORM COMPARISON OF NEW SEQUENCE AND STORED SEQUENCE
224
GO TO NEXT SEQUENCE IN DATABASE
212
SAME?
YES
NO
214
DISPLAY STORED SEQUENCE NAME TO USER
218
MORE SEQUENCES IN DATABASE?
YES
NO
220
END

FIGURE 2

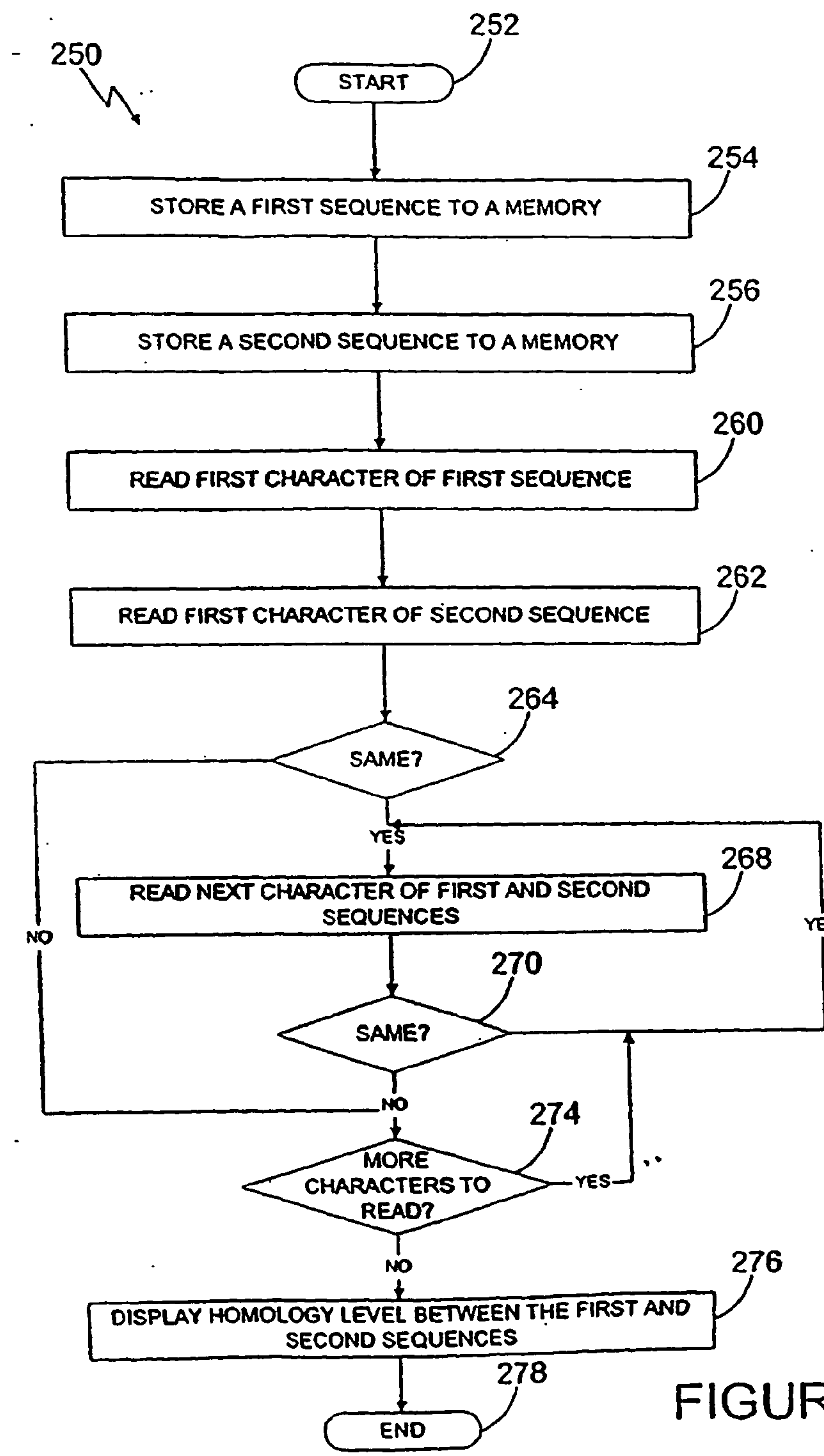

250
252
START
254
STORE A FIRST SEQUENCE TO A MEMORY
256
STORE A SECOND SEQUENCE TO A MEMORY
260
READ FIRST CHARACTER OF FIRST SEQUENCE
262
READ FIRST CHARACTER OF SECOND SEQUENCE
264
SAME?
NO
YES
268
READ NEXT CHARACTER OF FIRST AND SECOND SEQUENCES
YES
270
SAME?
NO
274
MORE CHARACTERS TO READ?
YES
NO
276
DISPLAY HOMOLOGY LEVEL BETWEEN THE FIRST AND SECOND SEQUENCES
278
END

FIGURE 3

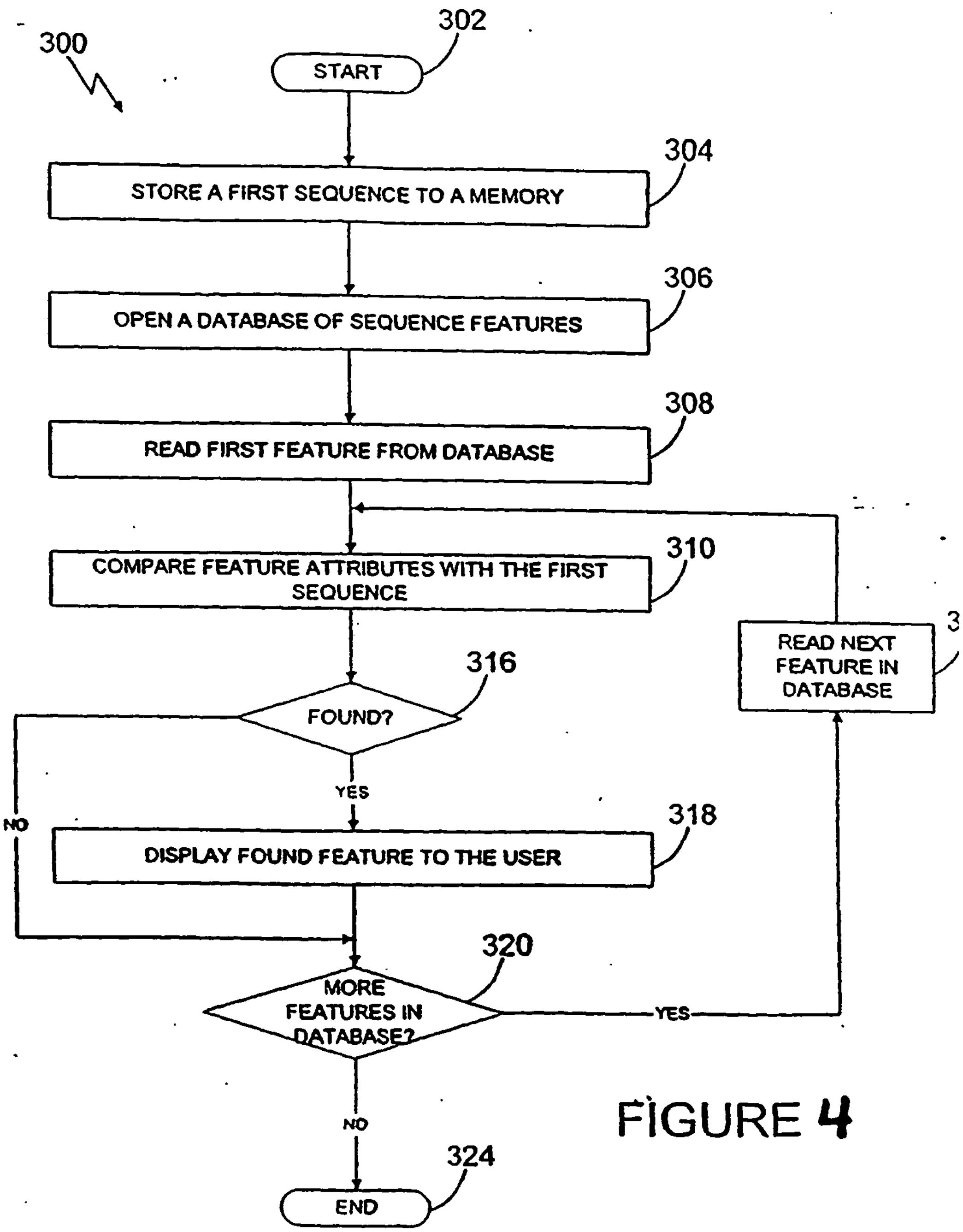

300
302
START
304
STORE A FIRST SEQUENCE TO A MEMORY
306
OPEN A DATABASE OF SEQUENCE FEATURES
308
READ FIRST FEATURE FROM DATABASE
310
COMPARE FEATURE ATTRIBUTES WITH THE FIRST SEQUENCE
316
FOUND?
YES
NO
318
DISPLAY FOUND FEATURE TO THE USER
320
MORE FEATURES IN DATABASE?
YES
READ NEXT FEATURE IN DATABASE
NO
324
END

FIGURE 4

FIGURE 5

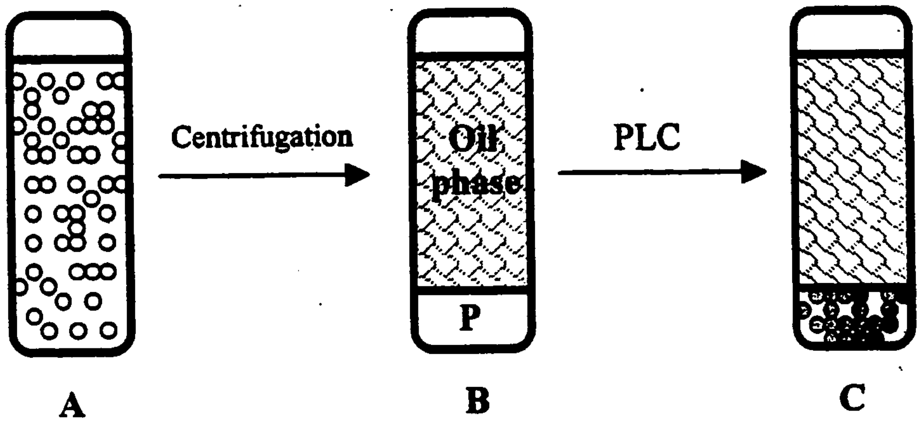


Figure 6

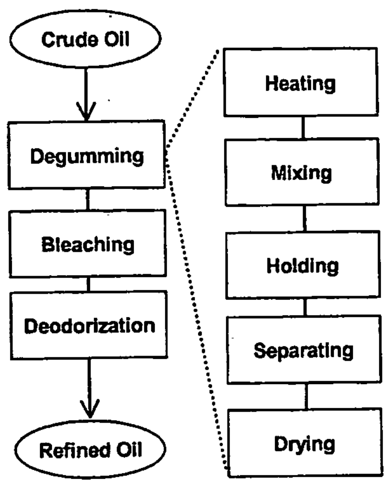

Crude Oil
Degumming
Bleaching
Deodorization
Refined Oil
Heating
Mixing
Holding
Separating
Drying

Figure 7

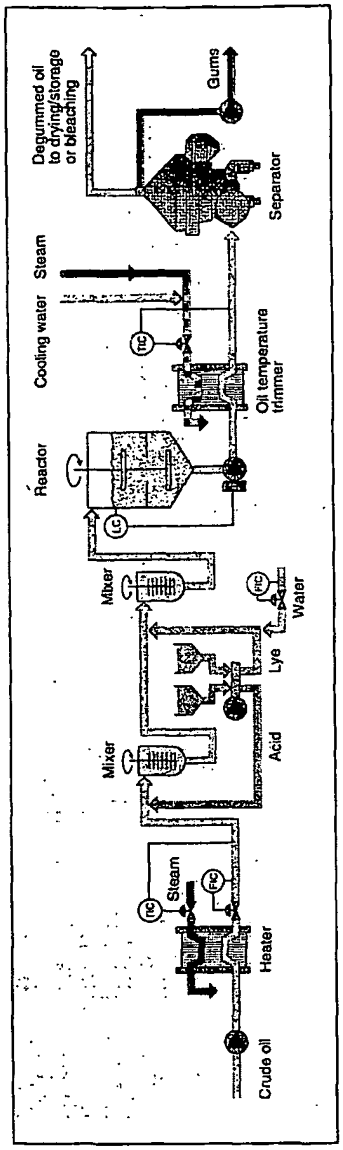

Crude oil
Heater
Steam
Mixer
Acid
Lye
Water
Mixer
Reactor
Oil temperature trimmer
Cooling water
Steam
Separator
Degummed oil to drying/storage or bleaching
Gums

Figure 8

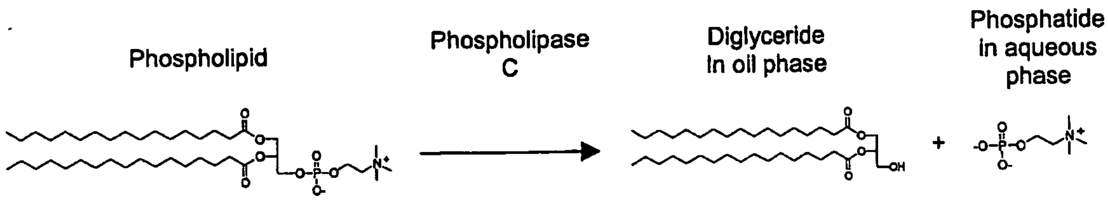

Phospholipid
Phospholipase
C
Diglyceride
in oil phase
Phosphatide
in aqueous
phase

Figure 9

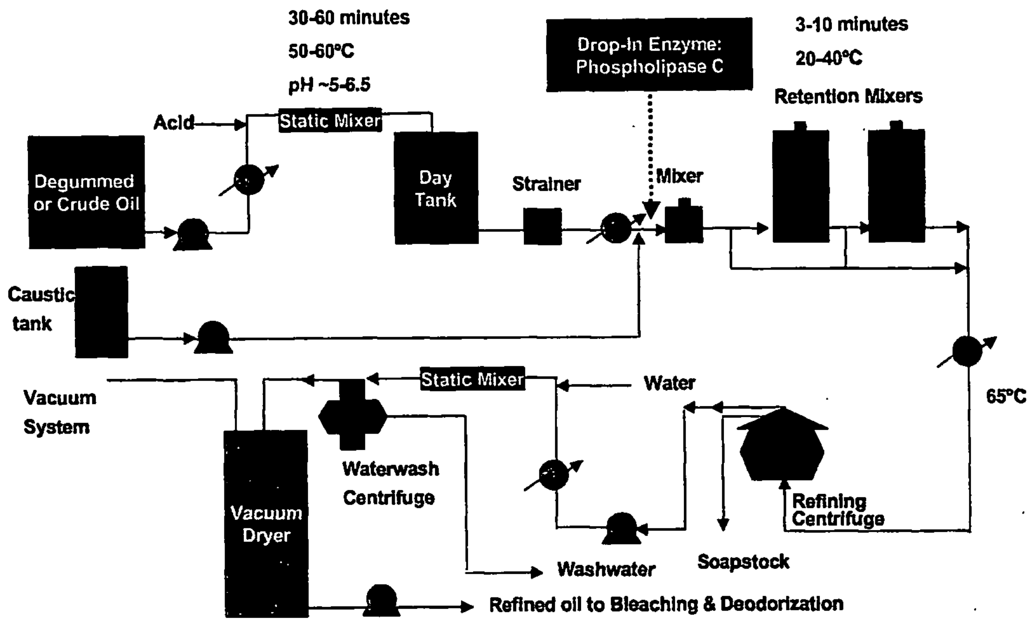
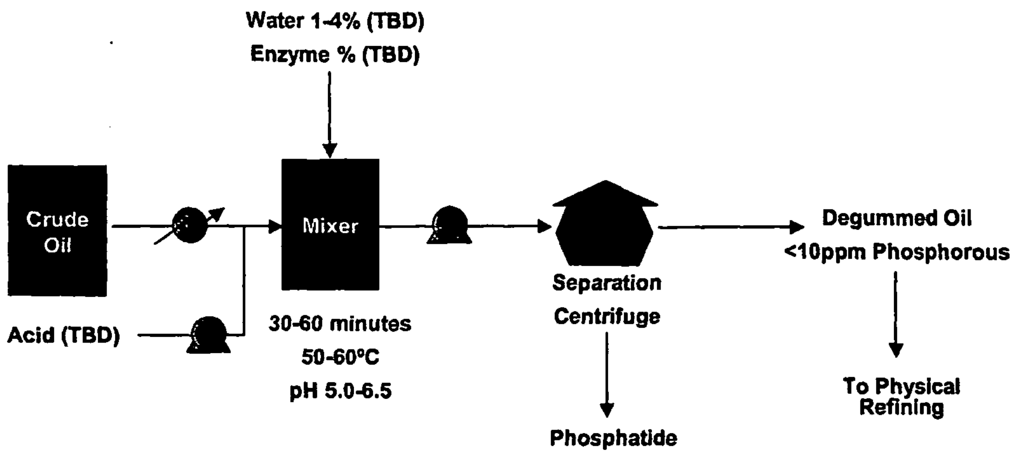

30-60 minutes
50-60°C
pH ~5-6.5
Drop-In Enzyme: Phospholipase C
3-10 minutes
20-40°C
Retention Mixers
Acid
Static Mixer
Degummed or Crude Oil
Day Tank
Strainer
Mixer
Caustic tank
Vacuum System
Static Mixer
Water
65°C
Waterwash Centrifuge
Vacuum Dryer
Refining Centrifuge
Washwater
Soapstock
Refined oil to Bleaching & Deodorization

Figure 10


Water 1-4% (TBD)
Enzyme % (TBD)
Crude Oil
Mixer
Acid (TBD)
30-60 minutes
50-60°C
pH 5.0-6.5
Separation Centrifuge
Phosphatide
Degummed Oil
<10ppm Phosphorous
To Physical Refining

SEQUENCE LISTING

<110> Svetlana Gramatikova, Nelson Barton
Geoff Hazlewood, David Lam

<120> PHOSPHOLIPASES, NUCLEIC ACIDS ENCODING THEM AND METHODS FOR MAKING AND USING THEM

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agtacatatg cttctcactt ttatgatccg gatactggaa caacatatat tccttttgcg 360
aaacatgcaa aagaaacagg cgcaaaatat tttaaccttg ctggtcaagc ataccaaaat 420
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tattggaatt ggaaaggagc aaacccagaa gattggattg aaggagcagc ggtagcagct 660
aaacaagatt atcctggcgt tgtgaacgat acgacaaaag attggtttgt aaaagcagcc 720
gtatctcaag aatatgcaga taaatggcgt gcggaagtaa caccggtgac aggaaagcgt 780
ttaatggaag cgcagcgcgt tacagctggt tatattcatt tgtggtttga tacgtatgta 840
aatcgctaa 849

<210> 2
<211> 282
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(24)

<400> 2
Met Lys Lys Lys Val Leu Ala Leu Ala Ala Met Val Ala Leu Ala Ala
1 5 10 15
Pro Val Gln Ser Val Val Phe Ala Gln Thr Asn Asn Ser Glu Ser Pro
20 25 30
Ala Pro Ile Leu-Arg Trp Ser Ala Glu Asp Lys His Asn Glu Gly Ile
35 40 45
Asn Ser His Leu Trp Ile Val Asn Arg Ala Ile Asp Ile Met Ser Arg
50 55 60
Asn Thr Thr Ile Val Asn Pro Asn Glu Thr Ala Leu Leu Asn Glu Trp
65 70 75 80
Arg Ala Asp Leu Glu Asn Gly Ile Tyr Ser Ala Asp Tyr Glu Asn Pro
85 90 95
Tyr Tyr Asp Asn Ser Thr Tyr Ala Ser His Phe Tyr Asp Pro Asp Thr
100 105 110
Gly Thr Thr Tyr Ile Pro Phe Ala Lys His Ala Lys Glu Thr Gly Ala
115 120 125
Lys Tyr Phe Asn Leu Ala Gly Gln Ala Tyr Gln Asn Gln Asp Met Gln
130 135 140
Gln Ala Phe Phe Tyr Leu Gly Leu Ser Leu His Tyr Leu Gly Asp Val
145 150 155 160
Asn Gln Pro Met His Ala Ala Asn Phe Thr Asn Leu Ser Tyr Pro Met
165 170 175
Gly Phe His Ser Lys Tyr Glu Asn Phe Val Asp Thr Ile Lys Asn Asn
180 185 190
Tyr Ile Val Ser Asp Ser Asn Gly Tyr Trp Asn Trp Lys Gly Ala Asn
195 200 205
Pro Glu Asp Trp Ile Glu Gly Ala Ala Val Ala Ala Lys Gln Asp Tyr
210 215 220
Pro Gly Val Val Asn Asp Thr Thr Lys Asp Trp Phe Val Lys Ala Ala
225 230 235 240
Val Ser Gln Glu Tyr Ala Asp Lys Trp Arg Ala Glu Val Thr Pro Val
245 250 255
Thr Gly Lys Arg Leu Met Glu Ala Gln Arg Val Thr Ala Gly Tyr Ile
260 265 270
His Leu Trp Phe Asp Thr Tyr Val Asn Arg
275 280

<210> 3
<211> 852
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 3
atgaaaagaa aaattttagc tatagcttcc gtaattgctt taacagctcc tatccaaagt 60
gtggcgtttg cgcatgaaaa tggtcaccaa gatccaccaa ttgctctaaa gtggtcagca 120
gaatctatac ataatgaagg agtaagttct catttatgga ttgtaaacag agccattgat 180
attatgtccc aaaatacgac tgttgtgaag caaaatgaga cagctctatt aaatgaatgg 240
cgtacggatc tagagaaagg catttactct gcggattatg aaaacccata ctatgataat 300
tccacattcg cttcacactt ctatgatcct gattcaggaa aaacgtatat tccatttgct 360
aaacaagcaa agcaaacagg agcgaaatat tttaaattag ctggtgaagc ttatcaaaat 420
aaagatctga aaaacgcatt cttttattta ggattatcac ttcactattt aggggatgtc 480
aaccaaccaa tgcatgcagc aaactttact aatatttcgc atccatttgg cttccactca 540
aaatatgaaa atttcgttga tacagtgaaa gacaattata gagtaacgga tggaaatggc 600

```
tattggaatt ggcaaagtgc aaatccagaa gagtgggttc atgcatcagc atcagcagca      660
aaagctgatt ttccatcaat tgttaatgat aagacgaaaa attggttcct aaaagcagct      720
gtatcacaag actctgctga taaatggcgt gcagaagtaa caccgataac aggaaaacgt      780
ttaatggaag cgcagcgtgt tacagctgga tatatccatt tatggtttga tacgtacgtg      840
aataacaaat aa                                                          852
```

<210> 4
<211> 283
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(24)

<400> 4

```
Met Lys Arg Lys Ile Leu Ala Ile Ala Ser Val Ile Ala Leu Thr Ala
 1               5                  10                  15
Pro Ile Gln Ser Val Ala Phe Ala His Glu Asn Gly His Gln Asp Pro
            20                  25                  30
Pro Ile Ala Leu Lys Trp Ser Ala Glu Ser Ile His Asn Glu Gly Val
        35                  40                  45
Ser Ser His Leu Trp Ile Val Asn Arg Ala Ile Asp Ile Met Ser Gln
    50                  55                  60
Asn Thr Thr Val Val Lys Gln Asn Glu Thr Ala Leu Leu Asn Glu Trp
65                  70                  75                  80
Arg Thr Asp Leu Glu Lys Gly Ile Tyr Ser Ala Asp Tyr Glu Asn Pro
                85                  90                  95
Tyr Tyr Asp Asn Ser Thr Phe Ala Ser His Phe Tyr Asp Pro Asp Ser
            100                 105                 110
Gly Lys Thr Tyr Ile Pro Phe Ala Lys Gln Ala Lys Gln Thr Gly Ala
        115                 120                 125
Lys Tyr Phe Lys Leu Ala Gly Glu Ala Tyr Gln Asn Lys Asp Leu Lys
    130                 135                 140
Asn Ala Phe Phe Tyr Leu Gly Leu Ser Leu His Tyr Leu Gly Asp Val
145                 150                 155                 160
Asn Gln Pro Met His Ala Ala Asn Phe Thr Asn Ile Ser His Pro Phe
                165                 170                 175
Gly Phe His Ser Lys Tyr Glu Asn Phe Val Asp Thr Val Lys Asp Asn
            180                 185                 190
Tyr Arg Val Thr Asp Gly Asn Gly Tyr Trp Asn Trp Gln Ser Ala Asn
        195                 200                 205
Pro Glu Glu Trp Val His Ala Ser Ala Ser Ala Ala Lys Ala Asp Phe
    210                 215                 220
Pro Ser Ile Val Asn Asp Lys Thr Lys Asn Trp Phe Leu Lys Ala Ala
225                 230                 235                 240
Val Ser Gln Asp Ser Ala Asp Lys Trp Arg Ala Glu Val Thr Pro Ile
                245                 250                 255
Thr Gly Lys Arg Leu Met Glu Ala Gln Arg Val Thr Ala Gly Tyr Ile
            260                 265                 270
His Leu Trp Phe Asp Thr Tyr Val Asn Asn Lys
        275                 280
```

<210> 5
<211> 843

<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 5
atgaaaagaa aaattttagc tatagcttct gtaattgctt taacagctcc tattcaaagt 60
gtggcgtttg cgcatgaatc tgatgggcct attgctttaa gatggtcagc ggaatctgta 120
cataatgaag gagtaagttc tcatttatgg attgtaaaca gagcaattga tattatgtcc 180
caaaatacga ctgtggtgaa gcaaaatgag acagctctat taaatgaatg gcgtacgaat 240
ttggaggaag gtatttattc tgcagattat aaaaacccat actatgataa ttccacattc 300
gcttcacact tctatgatcc tgattcagaa aaaacgtata ttccatttgc taaacaagca 360
aagcaaacgg gagcaaagta ttttaaatta gctggtgaag cttatcaaaa taaagatctg 420
aaaaatgcat tcttttattt aggattatca cttcattatt taggggatgt caatcaacca 480
atgcatgcag caaactttac taacatttcg catccatttg gcttccactc aaaatatgaa 540
aacttcgttg atacagtgaa agacaattat agagtaacag atggagatgg ctattggaat 600
tggaaaagtg caaatccaga agagtgggtt catgcatcag catcagcagc aaaagctgat 660
ttcccatcaa ttgttaatga taatacgaaa agttggttcc taaaagcagc ggtatcacaa 720
gactctgctg acaaatggcg tgctgaagta acaccggtaa caggaaaacg tttaatggaa 780
gcacagcgta ttacagctgg atatattcat ttatggtttg atacgtacgt gaataacaaa 840
taa 843

<210> 6
<211> 280
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(24)

<400> 6
Met Lys Arg Lys Ile Leu Ala Ile Ala Ser Val Ile Ala Leu Thr Ala
1 5 10 15
Pro Ile Gln Ser Val Ala Phe Ala His Glu Ser Asp Gly Pro Ile Ala
20 25 30
Leu Arg Trp Ser Ala Glu Ser Val His Asn Glu Gly Val Ser Ser His
35 40 45
Leu Trp Ile Val Asn Arg Ala Ile Asp Ile Met Ser Gln Asn Thr Thr
50 55 60
Val Val Lys Gln Asn Glu Thr Ala Leu Leu Asn Glu Trp Arg Thr Asn
65 70 75 80
Leu Glu Glu Gly Ile Tyr Ser Ala Asp Tyr Lys Asn Pro Tyr Tyr Asp
85 90 95
Asn Ser Thr Phe Ala Ser His Phe Tyr Asp Pro Asp Ser Glu Lys Thr
100 105 110
Tyr Ile Pro Phe Ala Lys Gln Ala Lys Gln Thr Gly Ala Lys Tyr Phe
115 120 125
Lys Leu Ala Gly Glu Ala Tyr Gln Asn Lys Asp Leu Lys Asn Ala Phe
130 135 140
Phe Tyr Leu Gly Leu Ser Leu His Tyr Leu Gly Asp Val Asn Gln Pro
145 150 155 160
Met His Ala Ala Asn Phe Thr Asn Ile Ser His Pro Phe Gly Phe His
165 170 175

```
Ser Lys Tyr Glu Asn Phe Val Asp Thr Val Lys Asp Asn Tyr Arg Val
            180                 185                 190
Thr Asp Gly Asp Gly Tyr Trp Asn Trp Lys Ser Ala Asn Pro Glu Glu
        195                 200                 205
Trp Val His Ala Ser Ala Ser Ala Ala Lys Ala Asp Phe Pro Ser Ile
    210                 215                 220
Val Asn Asp Asn Thr Lys Ser Trp Phe Leu Lys Ala Ala Val Ser Gln
225                 230                 235                 240
Asp Ser Ala Asp Lys Trp Arg Ala Glu Val Thr Pro Val Thr Gly Lys
                245                 250                 255
Arg Leu Met Glu Ala Gln Arg Ile Thr Ala Gly Tyr Ile His Leu Trp
            260                 265                 270
Phe Asp Thr Tyr Val Asn Asn Lys
        275                 280
```

<210> 7
<211> 963
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 7

```
gtgattactt tgataaaaaa atgtttatta gtattgacga tgactctatt gttaggggtt    60
ttcgtaccgc tgcagccatc acatgctact gaaaattatc caaatgattt taaactgttg   120
caacataatg tatttttatt gcctgaatca gtttcttatt ggggtcagga cgaacgtgca   180
gattatatga gtaatgcaga ttacttcaag ggacatgatg ctctgctctt aaatgagctt   240
tttgacaatg gaaattcgaa catgctgcta atgaacttat ccacggaata tccatatcaa   300
acgccagtgc ttggccgttc gatgagtgga tgggatgaaa ctagaggaag ctattctaat   360
tttgtacccg aagatggcgg tgtagcaatt atcagtaaat ggccaatcgt ggagaaaata   420
cagcatgttt acgcgaatgg ttgcggtgca gactattatg caaataaagg atttgtttat   480
gcaaaagtac aaaaagggga taaattctat catcttatca gcactcatgc tcaagccgaa   540
gatactgggt gtgatcaggg tgaaggagca gaaattcgtc attcacagtt tcaagaaatc   600
aacgacttta ttaaaaataa aaacattccg aaagatgaag tggtatttat tggtggtgac   660
tttaatgtga tgaagagtga cacaacagag tacaatagca tgttatcaac attaaatgtc   720
aatgcgccta ccgaatattt agggcatagc tctacttggg acccagaaac gaacagcatt   780
acaggttaca attaccctga ttatgcgcca cagcatttag attatatttt tgtggaaaaa   840
gatcataaac aaccaagttc atgggtaaat gaaacgatta ctccgaagtc tccaacttgg   900
aaggcaatct atgagtataa tgattattcc gatcactatc ctgttaaagc atacgtaaaa   960
taa                                                                  963
```

<210> 8
<211> 320
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(29)

<400> 8

```
Met Ile Thr Leu Ile Lys Lys Cys Leu Leu Val Leu Thr Met Thr Leu
 1               5                  10                  15
Leu Leu Gly Val Phe Val Pro Leu Gln Pro Ser His Ala Thr Glu Asn
```

20 25 30
Tyr Pro Asn Asp Phe Lys Leu Leu Gln His Asn Val Phe Leu Leu Pro
35 40 45
Glu Ser Val Ser Tyr Trp Gly Gln Asp Glu Arg Ala Asp Tyr Met Ser
50 55 60
Asn Ala Asp Tyr Phe Lys Gly His Asp Ala Leu Leu Leu Asn Glu Leu
65 70 75 80
Phe Asp Asn Gly Asn Ser Asn Met Leu Leu Met Asn Leu Ser Thr Glu
85 90 95
Tyr Pro Tyr Gln Thr Pro Val Leu Gly Arg Ser Met Ser Gly Trp Asp
100 105 110
Glu Thr Arg Gly Ser Tyr Ser Asn Phe Val Pro Glu Asp Gly Gly Val
115 120 125
Ala Ile Ile Ser Lys Trp Pro Ile Val Glu Lys Ile Gln His Val Tyr
130 135 140
Ala Asn Gly Cys Gly Ala Asp Tyr Tyr Ala Asn Lys Gly Phe Val Tyr
145 150 155 160
Ala Lys Val Gln Lys Gly Asp Lys Phe Tyr His Leu Ile Ser Thr His
165 170 175
Ala Gln Ala Glu Asp Thr Gly Cys Asp Gln Gly Glu Gly Ala Glu Ile
180 185 190
Arg His Ser Gln Phe Gln Glu Ile Asn Asp Phe Ile Lys Asn Lys Asn
195 200 205
Ile Pro Lys Asp Glu Val Val Phe Ile Gly Gly Asp Phe Asn Val Met
210 215 220
Lys Ser Asp Thr Thr Glu Tyr Asn Ser Met Leu Ser Thr Leu Asn Val
225 230 235 240
Asn Ala Pro Thr Glu Tyr Leu Gly His Ser Ser Thr Trp Asp Pro Glu
245 250 255
Thr Asn Ser Ile Thr Gly Tyr Asn Tyr Pro Asp Tyr Ala Pro Gln His
260 265 270
Leu Asp Tyr Ile Phe Val Glu Lys Asp His Lys Gln Pro Ser Ser Trp
275 280 285
Val Asn Glu Thr Ile Thr Pro Lys Ser Pro Thr Trp Lys Ala Ile Tyr
290 295 300
Glu Tyr Asn Asp Tyr Ser Asp His Tyr Pro Val Lys Ala Tyr Val Lys
305 310 315 320

<210> 9
<211> 999
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 9
atgaaattac tgcgtgtctt tgtgtgcgtt tttgctttac tcagcgcaca cagcaaagcc 60
gatacactta aagtaatggc ttataatatt atgcaactaa acgtacaaga ttgggatcaa 120
gcaaatcgtg cacagcgctt gccaaacgtc atatctcaat taagtgacag tcctgatgtc 180
attcttatca gcgaagcgtt tagcagccaa tcagaatctg cgttagcgca acttgctcaa 240
ctttaccctt atcaaactcc caatgttggc gaagactgta gtggcgctgg ctggcaaagc 300
ttaacgggta actgctcgaa tagccccttt gtgatccgcg gtggagtggt gattttatct 360
aagtacccca tcattacgca aaaagcccat gtgtttaata acagcctgac tgatagttgg 420
gattatttag caaacaaagg tttcgcttat gttgaaatag aaaaacatgg caaacgttac 480
caccttattg gcacgcattt acaagcaacg catgatggcg acacagaagc tgagcatatt 540
gtgagaatgg gtcaattaca agagatacaa gatttcattc aaagcgagca aattcacact 600

```
tctgagccgg tcattatcgg cggtgatatg aacgtagagt ggagcaagca atctgaaatt      660
acagatatgc tcgaagtggt tcgcagccgt ctaattttca acacacctga agttggctct      720
ttctctgcaa aacacaactg gtttaccaaa gctaacgcct actatttcga ctacagctta      780
gagtataacg acacgctcga ttatgtactt tggcatgcag accataagca acccaccaat      840
accccagaaa tgttagtacg ttacccaaaa gcagagcgtg acttttactg gcgttactta      900
cgcggaaatt ggaacttacc ttctggccgt tattatcatg atggatacta taacgaactg      960
tctgatcact acccagtgca agttaacttt gaattttaa                             999

<210> 10
<211> 332
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(20)

<400> 10
Met Lys Leu Leu Arg Val Phe Val Cys Val Phe Ala Leu Leu Ser Ala
 1               5                   10                  15
His Ser Lys Ala Asp Thr Leu Lys Val Met Ala Tyr Asn Ile Met Gln
            20                  25                  30
Leu Asn Val Gln Asp Trp Asp Gln Ala Asn Arg Ala Gln Arg Leu Pro
        35                  40                  45
Asn Val Ile Ser Gln Leu Ser Asp Ser Pro Asp Val Ile Leu Ile Ser
    50                  55                  60
Glu Ala Phe Ser Ser Gln Ser Glu Ser Ala Leu Ala Gln Leu Ala Gln
65                  70                  75                  80
Leu Tyr Pro Tyr Gln Thr Pro Asn Val Gly Glu Asp Cys Ser Gly Ala
                85                  90                  95
Gly Trp Gln Ser Leu Thr Gly Asn Cys Ser Asn Ser Pro Phe Val Ile
            100                 105                 110
Arg Gly Gly Val Val Ile Leu Ser Lys Tyr Pro Ile Ile Thr Gln Lys
        115                 120                 125
Ala His Val Phe Asn Asn Ser Leu Thr Asp Ser Trp Asp Tyr Leu Ala
    130                 135                 140
Asn Lys Gly Phe Ala Tyr Val Glu Ile Glu Lys His Gly Lys Arg Tyr
145                 150                 155                 160
His Leu Ile Gly Thr His Leu Gln Ala Thr His Asp Gly Asp Thr Glu
                165                 170                 175
Ala Glu His Ile Val Arg Met Gly Gln Leu Gln Glu Ile Gln Asp Phe
            180                 185                 190
Ile Gln Ser Glu Gln Ile His Thr Ser Glu Pro Val Ile Ile Gly Gly
        195                 200                 205
Asp Met Asn Val Glu Trp Ser Lys Gln Ser Glu Ile Thr Asp Met Leu
    210                 215                 220
Glu Val Val Arg Ser Arg Leu Ile Phe Asn Thr Pro Glu Val Gly Ser
225                 230                 235                 240
Phe Ser Ala Lys His Asn Trp Phe Thr Lys Ala Asn Ala Tyr Tyr Phe
                245                 250                 255
Asp Tyr Ser Leu Glu Tyr Asn Asp Thr Leu Asp Tyr Val Leu Trp His
            260                 265                 270
Ala Asp His Lys Gln Pro Thr Asn Thr Pro Glu Met Leu Val Arg Tyr
        275                 280                 285
Pro Lys Ala Glu Arg Asp Phe Tyr Trp Arg Tyr Leu Arg Gly Asn Trp
```

```
                290                 295                 300
Asn Leu Pro Ser Gly Arg Tyr Tyr His Asp Gly Tyr Tyr Asn Glu Leu
305                 310                 315                 320
Ser Asp His Tyr Pro Val Gln Val Asn Phe Glu Phe
                325                 330

<210> 11
<211> 1041
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 11
atggcttcac aattcaggaa tctggttttt gaaggaggcg gtgtaaaggg aatcgcctat      60
atcggcgcca tgcaggtgct ggagcagcgc ggacatttgg agcacgttgt gagggtggga     120
ggaacaagtg caggggctat taacgctctc attttttcgc tgggctttac cattaaagag     180
cagcaggata ttctcaattc caccaacttc agggagttta tggacagctc tttcggattt     240
gtgcgaaact tcagaaggct ctggagtgaa ttcgggtgga accgcggtga tgtgttttcg     300
gagtgggcag gagagctggt gaaagagaaa ctcggcaaga agaacgccac cttcggcgat     360
ctgaaaaaag cgaagcgccc cgatctctac gttatcggaa ccaacctctc caccgggttt     420
tccgagactt tttcgcatga acgccacgcc aacatgccgc tggtggatgc ggtgcggatc     480
agcatgtcga tcccgctctt ttttgcggca cgcagacttg gcaaacgaag cgatgtgtat     540
gtggatggag gtgttatgct caactacccg gtaaagctgt tcgacaggga gaaatacatc     600
gatttggaga aggagaaaga ggcagcccgc tacgtggagt actacaatca agagaatgcc     660
cggtttctgc ttgagcggcc cggccgaagc ccgtacgttt acaaccggca gaccctaggc     720
ctgcggctcg actcgcagga agagatcggc ctgttccgtt acgatgagcc gctgaagggc     780
aaacagatca accgcttccc cgaatatgcc aaagccctga tcggtgcact gatgcaggtg     840
caggagaaca tccacctgaa aagcgacgac tggcagcgaa cgctctacat caacacgctg     900
gatgtgggta ccacagattt cgacattaat gacgagaaga aaaaagtgct ggtgaatgag     960
ggaatcaagg gagcggaaac ctacttccgc tggtttgagg atcccgaagc taaaccggtg    1020
aacaaggtgg atttggtctg a                                              1041

<210> 12
<211> 346
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 12
Met Ala Ser Gln Phe Arg Asn Leu Val Phe Glu Gly Gly Gly Val Lys
1               5                   10                  15
Gly Ile Ala Tyr Ile Gly Ala Met Gln Val Leu Glu Gln Arg Gly His
            20                  25                  30
Leu Glu His Val Val Arg Val Gly Gly Thr Ser Ala Gly Ala Ile Asn
        35                  40                  45
Ala Leu Ile Phe Ser Leu Gly Phe Thr Ile Lys Glu Gln Gln Asp Ile
    50                  55                  60
Leu Asn Ser Thr Asn Phe Arg Glu Phe Met Asp Ser Ser Phe Gly Phe
65                  70                  75                  80
Val Arg Asn Phe Arg Arg Leu Trp Ser Glu Phe Gly Trp Asn Arg Gly
                85                  90                  95
Asp Val Phe Ser Glu Trp Ala Gly Glu Leu Val Lys Glu Lys Leu Gly
            100                 105                 110
```

Lys Lys Asn Ala Thr Phe Gly Asp Leu Lys Lys Ala Lys Arg Pro Asp
115 120 125
Leu Tyr Val Ile Gly Thr Asn Leu Ser Thr Gly Phe Ser Glu Thr Phe
130 135 140
Ser His Glu Arg His Ala Asn Met Pro Leu Val Asp Ala Val Arg Ile
145 150 155 160
Ser Met Ser Ile Pro Leu Phe Phe Ala Ala Arg Arg Leu Gly Lys Arg
165 170 175
Ser Asp Val Tyr Val Asp Gly Gly Val Met Leu Asn Tyr Pro Val Lys
180 185 190
Leu Phe Asp Arg Glu Lys Tyr Ile Asp Leu Glu Lys Glu Lys Glu Ala
195 200 205
Ala Arg Tyr Val Glu Tyr Tyr Asn Gln Glu Asn Ala Arg Phe Leu Leu
210 215 220
Glu Arg Pro Gly Arg Ser Pro Tyr Val Tyr Asn Arg Gln Thr Leu Gly
225 230 235 240
Leu Arg Leu Asp Ser Gln Glu Glu Ile Gly Leu Phe Arg Tyr Asp Glu
245 250 255
Pro Leu Lys Gly Lys Gln Ile Asn Arg Phe Pro Glu Tyr Ala Lys Ala
260 265 270
Leu Ile Gly Ala Leu Met Gln Val Gln Glu Asn Ile His Leu Lys Ser
275 280 285
Asp Asp Trp Gln Arg Thr Leu Tyr Ile Asn Thr Leu Asp Val Gly Thr
290 295 300
Thr Asp Phe Asp Ile Asn Asp Glu Lys Lys Lys Val Leu Val Asn Glu
305 310 315 320
Gly Ile Lys Gly Ala Glu Thr Tyr Phe Arg Trp Phe Glu Asp Pro Glu
325 330 335
Ala Lys Pro Val Asn Lys Val Asp Leu Val
340 345

<210> 13
<211> 1038
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 13
atgacaacac aatttagaaa cttgatattt gaaggcggcg gtgtaaaagg tgttgcttac 60
attggcgcca tgcagattct cgaaaatcgt ggcgtgttgc aagatattca cagagtcgga 120
gggtgcagtg cgggtgcgat caacgcgctg atttttgcgc tgggttacac ggtccgtgag 180
caaaaagaga tcttacaagc cacggatttt aaccagttta tggataactc ttggggtgtt 240
attcgtgata ttcgcaggct tgctcgagac tttggctggc acaagggtga cttctttaat 300
agctggatag gtgatttgat tcatcgtcgt ttggggaatc gccgagcgac gttcaaagat 360
ctgcaaaagg ccaagcttcc tgatctttat gtcatcggta ctaatctgtc tacagggtat 420
gcagaggttt tttcagccga aagacacccc gatatggagc tagcgacagc ggtgcgtatc 480
tccatgtcga taccgctgtt ctttgcggcc gtgcgtcacg gtgaacgaca agatgtgtat 540
gtcgatgggg gtgttcaact taactatccg attaaactgt ttgatcggga gcgttacatt 600
gatctggtca aagatcccgg tgccgttcgg cgaacgggtt attacaacaa agaaaacgct 660
cgctttcagc ttgagcggcc gggccatagc ccctatgttt acaatcgcca gaccttgggt 720
ttgcgactgg atagtcgaga ggagataggg ctctttcgtt atgacgaacc cctcaagggc 780
aaacccatta agtccttcac tgactacgct cgacaacttt tcggtgcgtt gatgaatgca 840
caggaaaaca ttcatctaca tggcgatgat tggcgcgcca cggtctatat cgatacattg 900
gatgtgggta cgacggattt caatctttct gatgcaacca agcaagcact gattgagcaa 960
ggaattaacg gcaccgaaaa ttatttcgac tggtttgata atccgttaga gaagcctgtg 1020

aatagagtgg agtcatag 1038

<210> 14
<211> 345
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 14
Met Thr Thr Gln Phe Arg Asn Leu Ile Phe Glu Gly Gly Gly Val Lys
1 5 10 15
Gly Val Ala Tyr Ile Gly Ala Met Gln Ile Leu Glu Asn Arg Gly Val
20 25 30
Leu Gln Asp Ile His Arg Val Gly Gly Cys Ser Ala Gly Ala Ile Asn
35 40 45
Ala Leu Ile Phe Ala Leu Gly Tyr Thr Val Arg Glu Gln Lys Glu Ile
50 55 60
Leu Gln Ala Thr Asp Phe Asn Gln Phe Met Asp Asn Ser Trp Gly Val
65 70 75 80
Ile Arg Asp Ile Arg Arg Leu Ala Arg Asp Phe Gly Trp His Lys Gly
85 90 95
Asp Phe Phe Asn Ser Trp Ile Gly Asp Leu Ile His Arg Arg Leu Gly
100 105 110
Asn Arg Arg Ala Thr Phe Lys Asp Leu Gln Lys Ala Lys Leu Pro Asp
115 120 125
Leu Tyr Val Ile Gly Thr Asn Leu Ser Thr Gly Tyr Ala Glu Val Phe
130 135 140
Ser Ala Glu Arg His Pro Asp Met Glu Leu Ala Thr Ala Val Arg Ile
145 150 155 160
Ser Met Ser Ile Pro Leu Phe Phe Ala Ala Val Arg His Gly Glu Arg
165 170 175
Gln Asp Val Tyr Val Asp Gly Gly Val Gln Leu Asn Tyr Pro Ile Lys
180 185 190
Leu Phe Asp Arg Glu Arg Tyr Ile Asp Leu Val Lys Asp Pro Gly Ala
195 200 205
Val Arg Arg Thr Gly Tyr Tyr Asn Lys Glu Asn Ala Arg Phe Gln Leu
210 215 220
Glu Arg Pro Gly His Ser Pro Tyr Val Tyr Asn Arg Gln Thr Leu Gly
225 230 235 240
Leu Arg Leu Asp Ser Arg Glu Glu Ile Gly Leu Phe Arg Tyr Asp Glu
245 250 255
Pro Leu Lys Gly Lys Pro Ile Lys Ser Phe Thr Asp Tyr Ala Arg Gln
260 265 270
Leu Phe Gly Ala Leu Met Asn Ala Gln Glu Asn Ile His Leu His Gly
275 280 285
Asp Asp Trp Ala Arg Thr Val Tyr Ile Asp Thr Leu Asp Val Gly Thr
290 295 300
Thr Asp Phe Asn Leu Ser Asp Ala Thr Lys Gln Ala Leu Ile Glu Gln
305 310 315 320
Gly Ile Asn Gly Thr Glu Asn Tyr Phe Asp Trp Phe Asp Asn Pro Leu
325 330 335
Glu Lys Pro Val Asn Arg Val Glu Ser
340 345

<210> 15

<211> 1344
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 15
atgctggtca tcattcatgg ctggagcgat gaggcgggct cgttcaagac cctggccaga 60
cgtttggcca aggcgccacc cgagggcctc gggacgcagg tcacggaaat ccatctgggt 120
gattatgtgt ccctggatga ccaggtgacg ttcaatgatc tggtcgatgc catggccaga 180
gcctggagcg atcgtggtct gcccacggcc ccgcgcagcg tcgatgccgt cgtgcacagc 240
accggcggcc tggtgatccg cgactggctc acgcagctgt acacgccgga aacagccccc 300
attcgtcgcc tgctgatgct cgctccggcc aatttcggct cgccgctggc acacaccgga 360
cgcagcatga tcggccgggt caccaagggc tggaagggca cgcggctctt tgaaacgggc 420
aagcacattc tcaaagggct cgaactggcc agcccctacg cctgggcgct ggccgaacgc 480
gatctgttca gcgatcagaa ctattatggc gccgggcgca tcctgtgcac tgtcctggtg 540
ggcaacgccg gttatcgcgg catcagcgcc gtcgccaacc ggcccggcac ggacggcacc 600
gtgcgcgtca gcagcgccaa tctccaagcg gccaggatgc tgctcgattt cagcgccagt 660
ccacaggctg agccggaatt caccctgcac gacagcaccg cggaaattgc cttcggcatc 720
gccgacgagg aagaccacag caccatcgcc gccaaggatc gcggcccgcg caaggcagtc 780
acctgggaac tgattctcaa agccctgcag atcgaggatg caagctttgc tcaatggtgc 840
cggcagatgc aggagcattc cgcggccgtg acggaaacgg cggaaaagcg ccgcaatgtt 900
cactacaaca gcttccagaa taccgtcgtg cgcgtggtgg acaaccacgg tgccgccgtg 960
caggattatc tcatcgagtt ttacatgaat gatgatcgca aactccgcga tcagcgcctc 1020
acccagcgcc tgcaggagca ggtgattacc aacgtgcacg gctacggtga cgacaagtcc 1080
tatcgcagca tgctgatcaa ctgcacggag ctctatgcgc tgatgtccag accgcaggat 1140
cgcctgaaca tcagcatcac cgcctatccg gatctctcca agggactggt ggggtatcgc 1200
acctacacgg acgaggatat cggttccctc tctctggatg cagcgcagat ccgaaagctc 1260
tttaagccgc accgtaccct gttgatgaca ctgtgcctgc aacgctatca gaaagatgat 1320
gtgttccgat tcagggatgt ttga 1344

<210> 16
<211> 447
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 16
Met Leu Val Ile Ile His Gly Trp Ser Asp Glu Ala Gly Ser Phe Lys
1 5 10 15
Thr Leu Ala Arg Arg Leu Ala Lys Ala Pro Pro Glu Gly Leu Gly Thr
20 25 30
Gln Val Thr Glu Ile His Leu Gly Asp Tyr Val Ser Leu Asp Asp Gln
35 40 45
Val Thr Phe Asn Asp Leu Val Asp Ala Met Ala Arg Ala Trp Ser Asp
50 55 60
Arg Gly Leu Pro Thr Ala Pro Arg Ser Val Asp Ala Val Val His Ser
65 70 75 80
Thr Gly Gly Leu Val Ile Arg Asp Trp Leu Thr Gln Leu Tyr Thr Pro
85 90 95
Glu Thr Ala Pro Ile Arg Arg Leu Leu Met Leu Ala Pro Ala Asn Phe
100 105 110
Gly Ser Pro Leu Ala His Thr Gly Arg Ser Met Ile Gly Arg Val Thr
115 120 125

```
Lys Gly Trp Lys Gly Thr Arg Leu Phe Glu Thr Gly Lys His Ile Leu
    130                 135                 140
Lys Gly Leu Glu Leu Ala Ser Pro Tyr Ala Trp Ala Leu Ala Glu Arg
145                 150                 155                 160
Asp Leu Phe Ser Asp Gln Asn Tyr Tyr Gly Ala Gly Arg Ile Leu Cys
                165                 170                 175
Thr Val Leu Val Gly Asn Ala Gly Tyr Arg Gly Ile Ser Ala Val Ala
            180                 185                 190
Asn Arg Pro Gly Thr Asp Gly Thr Val Arg Val Ser Ser Ala Asn Leu
        195                 200                 205
Gln Ala Ala Arg Met Leu Leu Asp Phe Ser Ala Ser Pro Gln Ala Glu
    210                 215                 220
Pro Glu Phe Thr Leu His Asp Ser Thr Ala Glu Ile Ala Phe Gly Ile
225                 230                 235                 240
Ala Asp Glu Glu Asp His Ser Thr Ile Ala Ala Lys Asp Arg Gly Pro
                245                 250                 255
Arg Lys Ala Val Thr Trp Glu Leu Ile Leu Lys Ala Leu Gln Ile Glu
            260                 265                 270
Asp Ala Ser Phe Ala Gln Trp Cys Arg Gln Met Gln Glu His Ser Ala
        275                 280                 285
Ala Val Thr Glu Thr Ala Glu Lys Arg Arg Asn Val His Tyr Asn Ser
    290                 295                 300
Phe Gln Asn Thr Val Val Arg Val Val Asp Asn His Gly Ala Ala Val
305                 310                 315                 320
Gln Asp Tyr Leu Ile Glu Phe Tyr Met Asn Asp Asp Arg Lys Leu Arg
                325                 330                 335
Asp Gln Arg Leu Thr Gln Arg Leu Gln Glu Gln Val Ile Thr Asn Val
            340                 345                 350
His Gly Tyr Gly Asp Asp Lys Ser Tyr Arg Ser Met Leu Ile Asn Cys
        355                 360                 365
Thr Glu Leu Tyr Ala Leu Met Ser Arg Pro Gln Asp Arg Leu Asn Ile
    370                 375                 380
Ser Ile Thr Ala Tyr Pro Asp Leu Ser Lys Gly Leu Val Gly Tyr Arg
385                 390                 395                 400
Thr Tyr Thr Asp Glu Asp Ile Gly Ser Leu Ser Leu Asp Ala Ala Gln
                405                 410                 415
Ile Arg Lys Leu Phe Lys Pro His Arg Thr Leu Leu Met Thr Leu Cys
            420                 425                 430
Leu Gln Arg Tyr Gln Lys Asp Asp Val Phe Arg Phe Arg Asp Val
        435                 440                 445
```

<210> 17
<211> 1137
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 17

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atgaaaaaaa gccttcaaca acatcttgcc gctgacggca gcccaaagaa tattctttct      60
ctcgacgggg gaggaatcag aggggctttg acccttggtt ttctcaaaaa aatagaaagc     120
atcctgcagg aaaaacatgg gaaggactat ctcctttgcg atcactttga tttgatcggt     180
ggaacttcca caggctccat cattgcagca gcattggcta taggcatgac agtggaggaa     240
atcactaaaa tgtatatgga tctgggcgga aaaattttcg gcaagaaaag gagtttctgg     300
agaccctggg aaactgcgaa atacttgaaa gcaggatatg accacaaagc tcttgaaaag     360
agtctgaaag atgctttcca ggattttctt ttaggaagtg accaaattag aacaggtctt     420
```

```
tgtatagtag ccaaaagagc agataccaat agtatatggc cattgattaa ccaccccaaa     480
ggaaaattct atgattcaga acaaggcaaa aacaaaaata tccccttatg gcaggcagta     540
agggcgagta ccgctgctcc aacctatttc gctccacaat taatagatgt gggtgatggt     600
caaaaggctg cttttgtgga cggaggggta agcatggcca ataaccccgc attaaccctg     660
ttaaaagtgg ctacacttaa aggttttcct tttcattggc caatgggaga agacaaactg     720
accatagttt cagtaggcac cggatatagt gttttccaaa gacaaaaggg tgaaatcacc     780
aaagcttcct tattaacttg ggccaaaaac gtcccggaaa tgttgatgca ggatgcttct     840
tggcagaatc agaccatact tcagtggatt tctaaatccc ccactgcaca ttccatagat     900
atggaaatgg aagaccttag agatgacttt ctaggcggaa gaccactcat caaatacctc     960
aggtacaact tccccttgac agtaaatgat ctcaatggat tgaagcttgg gaaaagcttt    1020
acccaaaaag aggtcgaaga tttggtggaa atgagcaatg cacataaccg agaggagttg    1080
tataggattg gggagaaggc ggctgaaggg tcggtaaaaa aagaacattt tgaataa       1137
```

<210> 18
<211> 378
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 18

```
Met Lys Lys Ser Leu Gln Gln His Leu Ala Ala Asp Gly Ser Pro Lys
1               5                   10                  15
Asn Ile Leu Ser Leu Asp Gly Gly Gly Ile Arg Gly Ala Leu Thr Leu
            20                  25                  30
Gly Phe Leu Lys Lys Ile Glu Ser Ile Leu Gln Glu Lys His Gly Lys
        35                  40                  45
Asp Tyr Leu Leu Cys Asp His Phe Asp Leu Ile Gly Gly Thr Ser Thr
    50                  55                  60
Gly Ser Ile Ile Ala Ala Ala Leu Ala Ile Gly Met Thr Val Glu Glu
65                  70                  75                  80
Ile Thr Lys Met Tyr Met Asp Leu Gly Gly Lys Ile Phe Gly Lys Lys
                85                  90                  95
Arg Ser Phe Trp Arg Pro Trp Glu Thr Ala Lys Tyr Leu Lys Ala Gly
            100                 105                 110
Tyr Asp His Lys Ala Leu Glu Lys Ser Leu Lys Asp Ala Phe Gln Asp
        115                 120                 125
Phe Leu Leu Gly Ser Asp Gln Ile Arg Thr Gly Leu Cys Ile Val Ala
    130                 135                 140
Lys Arg Ala Asp Thr Asn Ser Ile Trp Pro Leu Ile Asn His Pro Lys
145                 150                 155                 160
Gly Lys Phe Tyr Asp Ser Glu Gln Gly Lys Asn Lys Asn Ile Pro Leu
                165                 170                 175
Trp Gln Ala Val Arg Ala Ser Thr Ala Ala Pro Thr Tyr Phe Ala Pro
            180                 185                 190
Gln Leu Ile Asp Val Gly Asp Gly Gln Lys Ala Ala Phe Val Asp Gly
        195                 200                 205
Gly Val Ser Met Ala Asn Asn Pro Ala Leu Thr Leu Leu Lys Val Ala
    210                 215                 220
Thr Leu Lys Gly Phe Pro Phe His Trp Pro Met Gly Glu Asp Lys Leu
225                 230                 235                 240
Thr Ile Val Ser Val Gly Thr Gly Tyr Ser Val Phe Gln Arg Gln Lys
                245                 250                 255
Gly Glu Ile Thr Lys Ala Ser Leu Leu Thr Trp Ala Lys Asn Val Pro
            260                 265                 270
Glu Met Leu Met Gln Asp Ala Ser Trp Gln Asn Gln Thr Ile Leu Gln
```

```
        275                 280                 285
Trp Ile Ser Lys Ser Pro Thr Ala His Ser Ile Asp Met Glu Met Glu
    290                 295                 300
Asp Leu Arg Asp Asp Phe Leu Gly Gly Arg Pro Leu Ile Lys Tyr Leu
305                 310                 315                 320
Arg Tyr Asn Phe Pro Leu Thr Val Asn Asp Leu Asn Gly Leu Lys Leu
                325                 330                 335
Gly Lys Ser Phe Thr Gln Lys Glu Val Glu Asp Leu Val Glu Met Ser
            340                 345                 350
Asn Ala His Asn Arg Glu Glu Leu Tyr Arg Ile Gly Glu Lys Ala Ala
        355                 360                 365
Glu Gly Ser Val Lys Lys Glu His Phe Glu
    370                 375
```

<210> 19
<211> 1248
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 19

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atgaaaaaga caacgttagt tttggctcta ttgatgccat ttggtgccgc ctccgcacaa     60
gacaatagta tgactccaga agcaatcaca tcagctcaag tcgcacaaac acaatcagcc    120
tccacctata cctacgttag gtgttggtat cgaacagacg caagccatga ttcaccagca    180
accgactggg agtgggctag aaaggaaaac ggagactatt acaccattga cggttactgg    240
tggtcatcga tctcctttaa aaatatgttc tatagcgaga ctcctcaaca agagatcaag    300
cagcgttgtg tagacacctt ggatgttcag cacgacaaag ccgacatcac ctactttgcc    360
gctgacaacc gcttctctta caaccattct atctggacta acgatcacgg ctttcaagcg    420
aaccaaatca accgaatagt cgcttttggc gatagtcttt cagacacggg caacctattt    480
aatgggtcac aatggatttt ccctaaccct aattcttggt tcttgggtca cttctctaac    540
ggcttcgttt ggactgaata cttggctaac gctaagggcg ttccactcta taactgggct    600
gtgggtggcg cagcaggaac caaccaatat gtcgctctaa ctggtgtcta tgatcaggtc    660
acttcgtacc tgacttacat gaagatggcg aaaaattatc gcccagagaa cacactattc    720
acattagagt ttggattgaa tgactttatg aattacggac gtgaagtagc tgatgtaaaa    780
gctgacttta gtagcgcact gattcgcctc accgacgctg gcgcaaaaaa cattctgttg    840
ttcaccctac cagatgcgac caaagcccct cagtttaagt actcaacggc ccaagaaatc    900
gagacagttc gtggcaagat tctggcgttc aaccagttca tcaaagaaca agcagagtac    960
tatcaaagca aaggtgacaa cgtgatccta tttgatgcgc acgctctatt ctctagcatc   1020
accagcgacc cacaaaaaca cgggttcaga aacgcaaaag atgcctgcct agatattaat   1080
cgtagtgcat ctcaagacta cctatacagc catagtctga ccaacgactg tgcaacctat   1140
ggttctgata gctatgtatt ttggggcgta acacacccaa ccacagcaac tcataaatac   1200
atcgcaacgc atatactgat gaattcaatg tcgaccttcg acttttaa                 1248
```

<210> 20
<211> 415
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(19)

<400> 20

```
Met Lys Lys Thr Thr Leu Val Leu Ala Leu Leu Met Pro Phe Gly Ala
1               5                   10                  15
Ala Ser Ala Gln Asp Asn Ser Met Thr Pro Glu Ala Ile Thr Ser Ala
            20                  25                  30
Gln Val Ala Gln Thr Gln Ser Ala Ser Thr Tyr Thr Tyr Val Arg Cys
        35                  40                  45
Trp Tyr Arg Thr Asp Ala Ser His Asp Ser Pro Ala Thr Asp Trp Glu
    50                  55                  60
Trp Ala Arg Lys Glu Asn Gly Asp Tyr Tyr Thr Ile Asp Gly Tyr Trp
65                  70                  75                  80
Trp Ser Ser Ile Ser Phe Lys Asn Met Phe Tyr Ser Glu Thr Pro Gln
                85                  90                  95
Gln Glu Ile Lys Gln Arg Cys Val Asp Thr Leu Asp Val Gln His Asp
            100                 105                 110
Lys Ala Asp Ile Thr Tyr Phe Ala Ala Asp Asn Arg Phe Ser Tyr Asn
        115                 120                 125
His Ser Ile Trp Thr Asn Asp His Gly Phe Gln Ala Asn Gln Ile Asn
    130                 135                 140
Arg Ile Val Ala Phe Gly Asp Ser Leu Ser Asp Thr Gly Asn Leu Phe
145                 150                 155                 160
Asn Gly Ser Gln Trp Ile Phe Pro Asn Pro Asn Ser Trp Phe Leu Gly
                165                 170                 175
His Phe Ser Asn Gly Phe Val Trp Thr Glu Tyr Leu Ala Asn Ala Lys
            180                 185                 190
Gly Val Pro Leu Tyr Asn Trp Ala Val Gly Gly Ala Ala Gly Thr Asn
        195                 200                 205
Gln Tyr Val Ala Leu Thr Gly Val Tyr Asp Gln Val Thr Ser Tyr Leu
    210                 215                 220
Thr Tyr Met Lys Met Ala Lys Asn Tyr Arg Pro Glu Asn Thr Leu Phe
225                 230                 235                 240
Thr Leu Glu Phe Gly Leu Asn Asp Phe Met Asn Tyr Gly Arg Glu Val
                245                 250                 255
Ala Asp Val Lys Ala Asp Phe Ser Ser Ala Leu Ile Arg Leu Thr Asp
            260                 265                 270
Ala Gly Ala Lys Asn Ile Leu Leu Phe Thr Leu Pro Asp Ala Thr Lys
        275                 280                 285
Ala Pro Gln Phe Lys Tyr Ser Thr Ala Gln Glu Ile Glu Thr Val Arg
    290                 295                 300
Gly Lys Ile Leu Ala Phe Asn Gln Phe Ile Lys Glu Gln Ala Glu Tyr
305                 310                 315                 320
Tyr Gln Ser Lys Gly Asp Asn Val Ile Leu Phe Asp Ala His Ala Leu
                325                 330                 335
Phe Ser Ser Ile Thr Ser Asp Pro Gln Lys His Gly Phe Arg Asn Ala
            340                 345                 350
Lys Asp Ala Cys Leu Asp Ile Asn Arg Ser Ala Ser Gln Asp Tyr Leu
        355                 360                 365
Tyr Ser His Ser Leu Thr Asn Asp Cys Ala Thr Tyr Gly Ser Asp Ser
    370                 375                 380
Tyr Val Phe Trp Gly Val Thr His Pro Thr Thr Ala Thr His Lys Tyr
385                 390                 395                 400
Ile Ala Thr His Ile Leu Met Asn Ser Met Ser Thr Phe Asp Phe
                405                 410                 415
```

<210> 21
<211> 1716
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 21
atgcagcagc ataaattgag gaatttcaac aagggattga ccggcgtcgt attgagcgta 60
ttgacctcta ccagcgccat ggcttttaca caaatcggtg gcggcggcgc gattccgatg 120
ggccatgaat ggctcacgcg cagatccgca ctggaattat taaatgcaga ccatatcgtc 180
tccaacgacc cgctcgaccc acgcttgggc tggagccagg gcttggccaa aaatttggat 240
ctctccaatg cattgaacga agtgcagcgc atccagagcg ttaccaagac caacgcactt 300
tatgaaccac gctatgatga cgtgttttct gcgattgtcg gcgaacgctg ggtggacacg 360
gccggtttca acgttgcgaa ggctaccgtc ggtaaaatcg attgtttcag cgcggtcgcg 420
caagaacctg ccgatgttca gcaagaccat ttcatgcgtc gttacgatga cgtgggcgga 480
caaggtggcg ttaacgccgc acgccgcggg caacaacgtt tcatcaccca tttcatcaac 540
gccgcgatgg ccgaagaaaa aagcataaaa gcgtgggacg gcggtggata ctccacgctg 600
gaaaaagtca gccacaatta tttcttgttt ggtcgcgctg tgcatttgtt ccaggattct 660
ttcagcccgg aacacaccgt gcgtctgccg caagacaact acgaaaaagt acgtcaggta 720
aaagcctatc tgtgttccga aggcgcagag caacatacgc ataacgcgca ggatgcgatc 780
agcttcacca gcggcgacgt tatctggaag aaaaacaccc gtctggatgc cggctggagc 840
acctacaaac ccagcaatat gaaacccgtt gccttggtgg cgatggaagc ctcgaaggac 900
ttgtgggccg ccttcattcg caccatggcc gcaccgcgca gcgagcgtcg cgccattgct 960
cagcaagagg cacaaacgct ggtaaacaac tggttgtcgt tcgacgaaca ggaaatgctg 1020
agctggtacg acgaagaaac tcatcgcgat cacacttacg tgctcgaacc cggccagaac 1080
ggccccggta tttccatgtt cgattgcatg gtgggtctgg gcgtgacgtc tggcagccag 1140
gctgcgcgtg tggccgaact ggatcaacaa cgtcgccagt gcttgttcaa cgtcaaggcc 1200
accaccggtt acagcgatct gaacgatccg cacatggata tcccgtataa ctggcaatgg 1260
acgtcgacca cgcagtggaa agtgccaagc gcgagctgga cgattccgca gttgccggcc 1320
gacgcaggca agaaagtgac gatcaaaaac gccatcaacg gcaatccgct ggtagcgccg 1380
gctggcgtca aacacaacag cgatatttat tccgcgccgg gtgaagccat cgaattcatt 1440
ttcgtcggtg actacaacaa tgagtcttat ctgcgctcga aaaaagatgc ggatttgttc 1500
ttgagctaca gtgcggtatc cggcaagggc ttgctgtaca acacaccgaa tcaggcaggt 1560
tatcgcgtga aaccggcggg cgtgctgtgg acgatcgaga acacctactg gaatgatttc 1620
ctgtggttca acagttcgaa caaccgcatc tacgtaagcg gcacgggcga tgccaacaag 1680
ttacattcac agtggatcat tgacggtctg aaataa 1716

<210> 22
<211> 571
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(28)

<400> 22
Met Gln Gln His Lys Leu Arg Asn Phe Asn Lys Gly Leu Thr Gly Val
1 5 10 15
Val Leu Ser Val Leu Thr Ser Thr Ser Ala Met Ala Phe Thr Gln Ile
20 25 30
Gly Gly Gly Gly Ala Ile Pro Met Gly His Glu Trp Leu Thr Arg Arg
35 40 45
Ser Ala Leu Glu Leu Leu Asn Ala Asp His Ile Val Ser Asn Asp Pro
50 55 60
Leu Asp Pro Arg Leu Gly Trp Ser Gln Gly Leu Ala Lys Asn Leu Asp
65 70 75 80

Leu Ser Asn Ala Leu Asn Glu Val Gln Arg Ile Gln Ser Val Thr Lys
85 90 95
Thr Asn Ala Leu Tyr Glu Pro Arg Tyr Asp Asp Val Phe Ser Ala Ile
100 105 110
Val Gly Glu Arg Trp Val Asp Thr Ala Gly Phe Asn Val Ala Lys Ala
115 120 125
Thr Val Gly Lys Ile Asp Cys Phe Ser Ala Val Ala Gln Glu Pro Ala
130 135 140
Asp Val Gln Gln Asp His Phe Met Arg Arg Tyr Asp Asp Val Gly Gly
145 150 155 160
Gln Gly Gly Val Asn Ala Ala Arg Arg Gly Gln Gln Arg Phe Ile Thr
165 170 175
His Phe Ile Asn Ala Ala Met Ala Glu Glu Lys Ser Ile Lys Ala Trp
180 185 190
Asp Gly Gly Gly Tyr Ser Thr Leu Glu Lys Val Ser His Asn Tyr Phe
195 200 205
Leu Phe Gly Arg Ala Val His Leu Phe Gln Asp Ser Phe Ser Pro Glu
210 215 220
His Thr Val Arg Leu Pro Gln Asp Asn Tyr Glu Lys Val Arg Gln Val
225 230 235 240
Lys Ala Tyr Leu Cys Ser Glu Gly Ala Glu Gln His Thr His Asn Ala
245 250 255
Gln Asp Ala Ile Ser Phe Thr Ser Gly Asp Val Ile Trp Lys Lys Asn
260 265 270
Thr Arg Leu Asp Ala Gly Trp Ser Thr Tyr Lys Pro Ser Asn Met Lys
275 280 285
Pro Val Ala Leu Val Ala Met Glu Ala Ser Lys Asp Leu Trp Ala Ala
290 295 300
Phe Ile Arg Thr Met Ala Ala Pro Arg Ser Glu Arg Arg Ala Ile Ala
305 310 315 320
Gln Gln Glu Ala Gln Thr Leu Val Asn Asn Trp Leu Ser Phe Asp Glu
325 330 335
Gln Glu Met Leu Ser Trp Tyr Asp Glu Glu Thr His Arg Asp His Thr
340 345 350
Tyr Val Leu Glu Pro Gly Gln Asn Gly Pro Gly Ile Ser Met Phe Asp
355 360 365
Cys Met Val Gly Leu Gly Val Thr Ser Gly Ser Gln Ala Ala Arg Val
370 375 380
Ala Glu Leu Asp Gln Gln Arg Arg Gln Cys Leu Phe Asn Val Lys Ala
385 390 395 400
Thr Thr Gly Tyr Ser Asp Leu Asn Asp Pro His Met Asp Ile Pro Tyr
405 410 415
Asn Trp Gln Trp Thr Ser Thr Thr Gln Trp Lys Val Pro Ser Ala Ser
420 425 430
Trp Thr Ile Pro Gln Leu Pro Ala Asp Ala Gly Lys Lys Val Thr Ile
435 440 445
Lys Asn Ala Ile Asn Gly Asn Pro Leu Val Ala Pro Ala Gly Val Lys
450 455 460
His Asn Ser Asp Ile Tyr Ser Ala Pro Gly Glu Ala Ile Glu Phe Ile
465 470 475 480
Phe Val Gly Asp Tyr Asn Asn Glu Ser Tyr Leu Arg Ser Lys Lys Asp
485 490 495
Ala Asp Leu Phe Leu Ser Tyr Ser Ala Val Ser Gly Lys Gly Leu Leu
500 505 510
Tyr Asn Thr Pro Asn Gln Ala Gly Tyr Arg Val Lys Pro Ala Gly Val
515 520 525
Leu Trp Thr Ile Glu Asn Thr Tyr Trp Asn Asp Phe Leu Trp Phe Asn

530 535 540
Ser Ser Asn Asn Arg Ile Tyr Val Ser Gly Thr Gly Asp Ala Asn Lys
545 550 555 560
Leu His Ser Gln Trp Ile Ile Asp Gly Leu Lys
565 570

<210> 23
<211> 1473
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 23
atgacgatcc gctcgaccga ctacgcgctg ctcgcgcagg agagctacca cgacagccag 60
gtcgatgctg acgtcaagct cgatggcatc tcctacaagg tattcgccac cacggacgac 120
cccctcaccg gcttccaggc caccgcttac cagcgccagg atacgggcga ggtggtcatc 180
gcctaccgcg gcacggaatt cgaccgcgaa cccgtgcgcg atggcggcgt cgacgcaggc 240
atggtgttgc ttggcgtcaa cgcccagtca cctgcatccg aggtattcac ccgcgaagtg 300
atcgaaaagg cgaagcacga agccgagctc aacgatcgcg agccgaagat caccgtcacc 360
gggcattccc tcggcggcac cctcgccgaa atcaatgccg cgaaatacgg cctccacggc 420
gaaaccttca atgcctacgg tgcggccagc ctcaagggca tccccgaggg cggcgacacg 480
gtgatcgacc atgtccgcgc cggcgatctc gtcagcgccg ccagcccgca ctacgggcag 540
gtgcgtgtgt acgcagctca gcaggatatc gataccctgc aacatgccgg ctaccgcgac 600
gacagtggca tcttcagcct gcgcaacccc atcaaggcca cggatttcga cgcccacgcg 660
atcgataact tcgtgcccaa cagcaagctg cttggccaat cgatcatcgc tcctgagaac 720
gaagcccgtt acgaagccca caagggcatg atcgatcgct atcgcgatga cgtggccgat 780
atccggaaag gcatctccgc tccctgggaa atccccaagg ccgtcggcga gctgaaggac 840
aagctcgaac acgaagcctt cgagctggcc ggcaagggca tcctcgccgt cgagcacggt 900
gtagccgagg tcgttcacga ggcgaaggaa gggttcgatc atctcaagga aggcttgcac 960
cacgtcaggg aagagatcag cgagggcatc cacgccgtgg aagagaaggc ttccagcgca 1020
tggcacaccc tcacccaccc gaaggaatgg ttcgagcacg acaaacctca agtgaatctc 1080
gaccatcccc agcatccaga caacgccttg ttcaagcagg cgcagggcgc ggtacacgcc 1140
ctcgatgcca cgcaaggccg cacgccagat aggacgagcg accagatcgc aggttctctg 1200
gtggtcgcgg cgcgacgcga tggtctcgag cgggtggacc gcgccgtgct cagcgatgac 1260
actagccggc tctacggcgt gcagggtgcg acggattcgc ccttgaagca gttcaccgag 1320
gtgaacacga cagtggcggc gcaaacgtca ctgcagcaaa gcagccaggc atggcagcag 1380
caagcagaga tcgcgcgaca gaaccaggca accagccagg ctcagcgcat ggaaccgcag 1440
gtgcccccgc aggcaccggc acatggcatg taa 1473

<210> 24
<211> 490
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 24
Met Thr Ile Arg Ser Thr Asp Tyr Ala Leu Leu Ala Gln Glu Ser Tyr
1 5 10 15
His Asp Ser Gln Val Asp Ala Asp Val Lys Leu Asp Gly Ile Ser Tyr
20 25 30
Lys Val Phe Ala Thr Thr Asp Asp Pro Leu Thr Gly Phe Gln Ala Thr
35 40 45
Ala Tyr Gln Arg Gln Asp Thr Gly Glu Val Val Ile Ala Tyr Arg Gly

```
    50                  55                  60
Thr Glu Phe Asp Arg Glu Pro Val Arg Asp Gly Gly Val Asp Ala Gly
65                  70                  75                  80
Met Val Leu Leu Gly Val Asn Ala Gln Ser Pro Ala Ser Glu Val Phe
                85                  90                  95
Thr Arg Glu Val-Ile Glu Lys Ala Lys His Glu Ala Glu Leu Asn Asp
            100                 105                 110
Arg Glu Pro Lys Ile Thr Val Thr Gly His Ser Leu Gly Gly Thr Leu
        115                 120                 125
Ala Glu Ile Asn Ala Ala Lys Tyr Gly Leu His Gly Glu Thr Phe Asn
    130                 135                 140
Ala Tyr Gly Ala Ala Ser Leu Lys Gly Ile Pro Glu Gly Gly Asp Thr
145                 150                 155                 160
Val Ile Asp His Val Arg Ala Gly Asp Leu Val Ser Ala Ala Ser Pro
                165                 170                 175
His Tyr Gly Gln Val Arg Val Tyr Ala Ala Gln Gln Asp Ile Asp Thr
            180                 185                 190
Leu Gln His Ala Gly Tyr Arg Asp Asp Ser Gly Ile Phe Ser Leu Arg
        195                 200                 205
Asn Pro Ile Lys Ala Thr Asp Phe Asp Ala His Ala Ile Asp Asn Phe
    210                 215                 220
Val Pro Asn Ser Lys Leu Leu Gly Gln Ser Ile Ile Ala Pro Glu Asn
225                 230                 235                 240
Glu Ala Arg Tyr Glu Ala His Lys Gly Met Ile Asp Arg Tyr Arg Asp
                245                 250                 255
Asp Val Ala Asp Ile Arg Lys Gly Ile Ser Ala Pro Trp Glu Ile Pro
            260                 265                 270
Lys Ala Val Gly Glu Leu Lys Asp Lys Leu Glu His Glu Ala Phe Glu
        275                 280                 285
Leu Ala Gly Lys Gly Ile Leu Ala Val Glu His Gly Val Ala Glu Val
    290                 295                 300
Val His Glu Ala Lys Glu Gly Phe Asp His Leu Lys Glu Gly Leu His
305                 310                 315                 320
His Val Arg Glu Glu Ile Ser Glu Gly Ile His Ala Val Glu Glu Lys
                325                 330                 335
Ala Ser Ser Ala Trp His Thr Leu Thr His Pro Lys Glu Trp Phe Glu
            340                 345                 350
His Asp Lys Pro Gln Val Asn Leu Asp His Pro Gln His Pro Asp Asn
        355                 360                 365
Ala Leu Phe Lys Gln Ala Gln Gly Ala Val His Ala Leu Asp Ala Thr
    370                 375                 380
Gln Gly Arg Thr Pro Asp Arg Thr Ser Asp Gln Ile Ala Gly Ser Leu
385                 390                 395                 400
Val Val Ala Ala Arg Arg Asp Gly Leu Glu Arg Val Asp Arg Ala Val
                405                 410                 415
Leu Ser Asp Asp Thr Ser Arg Leu Tyr Gly Val Gln Gly Ala Thr Asp
            420                 425                 430
Ser Pro Leu Lys Gln Phe Thr Glu Val Asn Thr Thr Val Ala Ala Gln
        435                 440                 445
Thr Ser Leu Gln Gln Ser Ser Gln Ala Trp Gln Gln Gln Ala Glu Ile
    450                 455                 460
Ala Arg Gln Asn Gln Ala Thr Ser Gln Ala Gln Arg Met Glu Pro Gln
465                 470                 475                 480
Val Pro Pro Gln Ala Pro Ala His Gly Met
                485                 490
```

<210> 25

<211> 1098
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 25
atgtgcgcca aagttaaagt agtcaaaata aagacaaaca caggcagccc aaacaaatac 60
cacttcaaga acctcgtctt cgaaggcggc ggcgtgaaag gcattgccta tgtgggagcc 120
cttaccaagc tcgacgagga aggcatcctt caaaacatta agcgcgtggc cggcacctca 180
gcaggagcaa tggtggccgt cctcgtcgga ttgggcttca ccgctaagga gataagcgac 240
atcctgtggg acatcaaatt ccagaacttt ttagacaact catggggcgt gatacgcaac 300
accaatcgtc tgctgacgga atacggctgg tataagggcg agtttttccg cgacctcatg 360
gctgattaca tcaaaagaaa gacagacgat ggcgagatta ctttcgggga gttggaggcc 420
atgagaaaag agggcaagcc cttcttggaa atccatctgg ttggctccga cctcacgaca 480
gggtattcca gagtgttcaa ctccaaaaac accccaaatg tgaaagtcgc cgatgccgcc 540
cgcatctcca tgtcgatacc gctgtttttc tccgctgtga gaggcgtgca aggcgacgac 600
cacctctatg tggacggtgg gcttttggac aactacgcca tcaagatttt cgaccagtcg 660
aaactcgttt cagacaaaaa caacaaaagg aagaccgagt attacaacag gctcaaccag 720
caagtgaacg cgaaagcaac gaaaagcaag acggaatctg tagagtatgt ctacaacaag 780
gagactttgg gcttccgctt ggatgccaaa gaggacatca acctcttcct caaccacgat 840
gatgcccctc aaaaagaaat caagagtttc ttctcttaca ccaaagcttt ggtttccacg 900
ctcatcgatt tccagaacaa tgtacacctg cacagcgacg actggcagcg tacggtctac 960
atcgacacac tcggtgtcag ctccattgac ttcggtctgt caaacacaac gaaacaagct 1020
cttgtcgatt cgggctacaa ctacaccaca gcctacctcg actggtacaa caacgacgag 1080
gataaagcca acaagtaa 1098

<210> 26
<211> 365
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 26
Met Cys Ala Lys Val Lys Val Val Lys Ile Lys Thr Asn Thr Gly Ser
1 5 10 15
Pro Asn Lys Tyr His Phe Lys Asn Leu Val Phe Glu Gly Gly Gly Val
20 25 30
Lys Gly Ile Ala Tyr Val Gly Ala Leu Thr Lys Leu Asp Glu Glu Gly
35 40 45
Ile Leu Gln Asn Ile Lys Arg Val Ala Gly Thr Ser Ala Gly Ala Met
50 55 60
Val Ala Val Leu Val Gly Leu Gly Phe Thr Ala Lys Glu Ile Ser Asp
65 70 75 80
Ile Leu Trp Asp Ile Lys Phe Gln Asn Phe Leu Asp Asn Ser Trp Gly
85 90 95
Val Ile Arg Asn Thr Asn Arg Leu Leu Thr Glu Tyr Gly Trp Tyr Lys
100 105 110
Gly Glu Phe Phe Arg Asp Leu Met Ala Asp Tyr Ile Lys Arg Lys Thr
115 120 125
Asp Asp Gly Glu Ile Thr Phe Gly Glu Leu Glu Ala Met Arg Lys Glu
130 135 140
Gly Lys Pro Phe Leu Glu Ile His Leu Val Gly Ser Asp Leu Thr Thr
145 150 155 160

```
Gly Tyr Ser Arg Val Phe Asn Ser Lys Asn Thr Pro Asn Val Lys Val
                165                 170                 175
Ala Asp Ala Ala Arg Ile Ser Met Ser Ile Pro Leu Phe Phe Ser Ala
            180                 185                 190
Val Arg Gly Val Gln Gly Asp Asp His Leu Tyr Val Asp Gly Gly Leu
        195                 200                 205
Leu Asp Asn Tyr Ala Ile Lys Ile Phe Asp Gln Ser Lys Leu Val Ser
    210                 215                 220
Asp Lys Asn Asn Lys Arg Lys Thr Glu Tyr Tyr Asn Arg Leu Asn Gln
225                 230                 235                 240
Gln Val Asn Ala Lys Ala Thr Lys Ser Lys Thr Glu Ser Val Glu Tyr
                245                 250                 255
Val Tyr Asn Lys Glu Thr Leu Gly Phe Arg Leu Asp Ala Lys Glu Asp
            260                 265                 270
Ile Asn Leu Phe Leu Asn His Asp Asp Ala Pro Gln Lys Glu Ile Lys
        275                 280                 285
Ser Phe Phe Ser Tyr Thr Lys Ala Leu Val Ser Thr Leu Ile Asp Phe
    290                 295                 300
Gln Asn Asn Val His Leu His Ser Asp Asp Trp Gln Arg Thr Val Tyr
305                 310                 315                 320
Ile Asp Thr Leu Gly Val Ser Ser Ile Asp Phe Gly Leu Ser Asn Thr
                325                 330                 335
Thr Lys Gln Ala Leu Val Asp Ser Gly Tyr Asn Tyr Thr Thr Ala Tyr
            340                 345                 350
Leu Asp Trp Tyr Asn Asn Asp Glu Asp Lys Ala Asn Lys
        355                 360                 365
```

<210> 27
<211> 1287
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 27

```
gtgtcgatta ccgtttaccg gaagccctcc ggcgggtttg gagcgatagt tcctcaagcg      60
aaaattgaga accttgtttt cgagggcggc ggaccaaagg gcctggtcta tgtcggcgcg     120
gtcgaggttc tcggcgaaag gggactgctg gaagggatcg caaatgtcgg cggcgcttca     180
gcaggcgcca tgaccgctct agccgtcggt ctgggactga gccccaggga aattcgcgcg     240
gtcgtcttta accagaacat tgcggacctc accgatatcg agaagaccgt cgagccgtcc     300
tccgggatta caggcatgtt caagagcgtg ttcaagaagg gttggcaggc ggtgcgcaac     360
gtaaccggca cctctgacga gcgcgggcgc gggctctatc gcggcgagaa gttgcgagcc     420
tggatcagag acctgattgc acagcgagtc gaggcggggc gctccgaggt cctgagccga     480
gccgacgccg atggacggaa cttctatgag aaagccgccg caaagaaggg cgccctgaca     540
tttgccgagc ttgatcgggt ggcgcaaatg gcgccgggcc tgcggcttcg ccgcctggcc     600
ttcaccggaa ccaacttcac gtcgaagaag ctcgaagtgt tcagtctgca cgagaccccg     660
gacatgccga tcgacgtcgc ggtacgcatc tccgcatcgt tgccatggtt tttcaaatcc     720
gtgaaatgga acggctccga atacatagat ggcggctgcc tgtcgaactt cccaatgccg     780
atattcgacg tcgatcccta tcgtggcgac gcatcgtcga aaatccggct cggcatcttc     840
ggccagaacc tcgcgacgct cggcttcaag gtcgacagcg aggaggagat ccgcgacatt     900
ctctggcgta gccccgagag cacgagcgac ggctttttcc aaggcatcct gtcaagcgtg     960
aaagcttctg cagaacactg ggtcgtcggc atcgacgtcg aaggcgccac ccgcgcgtcg    1020
aacgtggccg ttcacggcaa gtatgctcag cgaacgatcc agataccgga cctcggatat    1080
agcacgttca agttcgatct ttcggacgct gacaaggagc gcatggccga ggccggcgca    1140
aaggccacgc gggaatggct ggcgctgtac ttcgacgacg ccggaataga ggtcgaattt    1200
tctgatccga acgaattgcg cggccagttg tccgacgccg cattcgcaga cctcgaggat    1260
```

tcgtttcgag ccttgatcgc ggcctag 1287

<210> 28
<211> 428
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 28
Met Ser Ile Thr Val Tyr Arg Lys Pro Ser Gly Gly Phe Gly Ala Ile
1 5 10 15
Val Pro Gln Ala Lys Ile Glu Asn Leu Val Phe Glu Gly Gly Gly Pro
20 25 30
Lys Gly Leu Val Tyr Val Gly Ala Val Glu Val Leu Gly Glu Arg Gly
35 40 45
Leu Leu Glu Gly Ile Ala Asn Val Gly Gly Ala Ser Ala Gly Ala Met
50 55 60
Thr Ala Leu Ala Val Gly Leu Gly Leu Ser Pro Arg Glu Ile Arg Ala
65 70 75 80
Val Val Phe Asn Gln Asn Ile Ala Asp Leu Thr Asp Ile Glu Lys Thr
85 90 95
Val Glu Pro Ser Ser Gly Ile Thr Gly Met Phe Lys Ser Val Phe Lys
100 105 110
Lys Gly Trp Gln Ala Val Arg Asn Val Thr Gly Thr Ser Asp Glu Arg
115 120 125
Gly Arg Gly Leu Tyr Arg Gly Glu Lys Leu Arg Ala Trp Ile Arg Asp
130 135 140
Leu Ile Ala Gln Arg Val Glu Ala Gly Arg Ser Glu Val Leu Ser Arg
145 150 155 160
Ala Asp Ala Asp Gly Arg Asn Phe Tyr Glu Lys Ala Ala Ala Lys Lys
165 170 175
Gly Ala Leu Thr Phe Ala Glu Leu Asp Arg Val Ala Gln Met Ala Pro
180 185 190
Gly Leu Arg Leu Arg Arg Leu Ala Phe Thr Gly Thr Asn Phe Thr Ser
195 200 205
Lys Lys Leu Glu Val Phe Ser Leu His Glu Thr Pro Asp Met Pro Ile
210 215 220
Asp Val Ala Val Arg Ile Ser Ala Ser Leu Pro Trp Phe Phe Lys Ser
225 230 235 240
Val Lys Trp Asn Gly Ser Glu Tyr Ile Asp Gly Gly Cys Leu Ser Asn
245 250 255
Phe Pro Met Pro Ile Phe Asp Val Asp Pro Tyr Arg Gly Asp Ala Ser
260 265 270
Ser Lys Ile Arg Leu Gly Ile Phe Gly Gln Asn Leu Ala Thr Leu Gly
275 280 285
Phe Lys Val Asp Ser Glu Glu Glu Ile Arg Asp Ile Leu Trp Arg Ser
290 295 300
Pro Glu Ser Thr Ser Asp Gly Phe Phe Gln Gly Ile Leu Ser Ser Val
305 310 315 320
Lys Ala Ser Ala Glu His Trp Val Val Gly Ile Asp Val Glu Gly Ala
325 330 335
Thr Arg Ala Ser Asn Val Ala Val His Gly Lys Tyr Ala Gln Arg Thr
340 345 350
Ile Gln Ile Pro Asp Leu Gly Tyr Ser Thr Phe Lys Phe Asp Leu Ser
355 360 365

```
Asp Ala Asp Lys Glu Arg Met Ala Glu Ala Gly Ala Lys Ala Thr Arg
    370                 375                 380
Glu Trp Leu Ala Leu Tyr Phe Asp Asp Ala Gly Ile Glu Val Glu Phe
385                 390                 395                 400
Ser Asp Pro Asn Glu Leu Arg Gly Gln Leu Ser Asp Ala Ala Phe Ala
                405                 410                 415
Asp Leu Glu Asp Ser Phe Arg Ala Leu Ile Ala Ala
            420                 425
```

<210> 29
<211> 753
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 29

```
atgggaaacg gtgcagcagt tggttcgaat gataatggta gagaagaaag tgtttacgta      60
ctttctgtga tcgcctgtaa tgtttattat ttacaaaagt gtgaaggtgg ggcatcgcgt     120
gatagcgtga ttagagaaat caatagccaa actcaacctt taggatatga gattgtagca     180
gattctattc gtgatggtca tattggctct tttgcctgta agatggctgt ctttagaaat     240
aatggaaacg gcaattgtgt tttagcaatc aaagggactg atatgaataa tatcaatgac     300
ttggtgaatg acctaaccat gatattagga ggtattggtt ctgttgctgc aatccaacca     360
acgattaaca tggcacaaga actcatcgac caatatggag tgaatttgat tacaggtcac     420
tcccttggag gctacatgac tgagatcatc gccaccaatc gtggacttcc aggtattgca     480
ttttgcgcac caggttcaaa tggtcccatt gtaaaattag gtggacaaga gacacctggc     540
tttcacaatg tgaactttga acatgatcca gcaggtaacg ttatgacggg ggtttatact     600
catgtccaat ggagtattta tgtaggatgt gatggtatga ctcatggtat tgaaaatatg     660
gtgaattatt ttaaagataa aagagattta accaatcgca atattcaagg aagaagtgaa     720
agtcataata cgggttatta ttacccaaaa taa                                  753
```

<210> 30
<211> 250
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 30

```
Met Gly Asn Gly Ala Ala Val Gly Ser Asn Asp Asn Gly Arg Glu Glu
1               5                   10                  15
Ser Val Tyr Val Leu Ser Val Ile Ala Cys Asn Val Tyr Tyr Leu Gln
            20                  25                  30
Lys Cys Glu Gly Gly Ala Ser Arg Asp Ser Val Ile Arg Glu Ile Asn
        35                  40                  45
Ser Gln Thr Gln Pro Leu Gly Tyr Glu Ile Val Ala Asp Ser Ile Arg
    50                  55                  60
Asp Gly His Ile Gly Ser Phe Ala Cys Lys Met Ala Val Phe Arg Asn
65                  70                  75                  80
Asn Gly Asn Gly Asn Cys Val Leu Ala Ile Lys Gly Thr Asp Met Asn
                85                  90                  95
Asn Ile Asn Asp Leu Val Asn Asp Leu Thr Met Ile Leu Gly Gly Ile
            100                 105                 110
Gly Ser Val Ala Ala Ile Gln Pro Thr Ile Asn Met Ala Gln Glu Leu
        115                 120                 125
```

```
Ile Asp Gln Tyr Gly Val Asn Leu Ile Thr Gly His Ser Leu Gly Gly
    130                 135                 140
Tyr Met Thr Glu Ile Ile Ala Thr Asn Arg Gly Leu Pro Gly Ile Ala
145                 150                 155                 160
Phe Cys Ala Pro Gly Ser Asn Gly Pro Ile Val Lys Leu Gly Gly Gln
                165                 170                 175
Glu Thr Pro Gly Phe His Asn Val Asn Phe Glu His Asp Pro Ala Gly
            180                 185                 190
Asn Val Met Thr Gly Val Tyr Thr His Val Gln Trp Ser Ile Tyr Val
        195                 200                 205
Gly Cys Asp Gly Met Thr His Gly Ile Glu Asn Met Val Asn Tyr Phe
    210                 215                 220
Lys Asp Lys Arg Asp Leu Thr Asn Arg Asn Ile Gln Gly Arg Ser Glu
225                 230                 235                 240
Ser His Asn Thr Gly Tyr Tyr Tyr Pro Lys
                245                 250
```

<210> 31
<211> 1422
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 31

```
atgaaaaaga aattatgtac atgggctctc gtaacagcga tatcttctgg agttgttgcg      60
attccaaccg tagcatctgc ttgcggaatg ggtgaagtaa tgaaacagga ggatcaagag     120
cacaaacgtg tgaagagatg gtctgcggag catccgcacc atgctaatga aagcacgcac     180
ttatggattg ctcgaaatgc gattcaaatt atgagtcgta atcaagataa gacggttcaa     240
gaaaatgaat tacaattctt aaaaatacct gaatataagg agttatttga aagagggctt     300
tatgatgccg attatcttga tgagtttaac gatggaggta caggtacaat cggtattgat     360
gggctaatta aaggaggctg gaaatctcat ttctatgatc ctgatacgaa aaagaactat     420
aaaggagaag aagaaccaac agccctttcg caaggggata aatattttaa attagcagga     480
gattatttta agaaagaaga ttggaaacaa gctttctatt atttaggtgt tgcgacgcat     540
tacttcacag atgctactca gccaatgcat gctgctaatt ttacagctgt cgacatgagt     600
gcaataaagt ttcatagcgc ttttgaaaat tatgtaacga cagttcagac accgtttgaa     660
gtgaaggatg ataagggaac atataatttg gtcaattctg atgatccgaa gcagtggata     720
catgaaacag cgaaactcgc aaaagcagaa attatgaata ttactagtga taatattaaa     780
tctcaatata ataaaggaaa caaagatctt tggcaacaag aagttatgcc agctgtccag     840
aggagtttag agaaagcgca aagaaacacg gcgggattta ttcatttatg gtttaaaaca     900
tatgttggca aaactgcagc tgaagatatt gaaactacac aggtaaaaga ttctaatgga     960
gaagcaatac aagaacaaaa aaaatactac gttgtgccta gtgagttttt aaatagaggt    1020
ttgacctttg aggtatatgc ttcgaatgac tacgcactat tatctaatca cgtagatgat    1080
aataaagttc atggtacacc tgttcagttt gtttttgata aagagaataa cggaattgtt    1140
catcggggag aaagtgtact gctgaaaatg acgcaatcta actatgatga ttatgtattt    1200
cttaattact ctaatatgac aaattggtta catcttgcga aacgaaaaac aaatactgca    1260
cagtttaaag tgtatccaaa tccggataac tcatctgaat atttcctata tacagatgga    1320
tacccggtaa attatcaaga aaatggtaat gggaagagct ggattgagtt aggaaagaaa    1380
acggataaac cgaaagcgtg gaaatttcaa caggcagaat aa                         1422
```

<210> 32
<211> 473
<212> PRT
<213> Unknown

<220>

<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(20)

<400> 32
```
Met Lys Lys Lys Leu Cys Thr Trp Ala Leu Val Thr Ala Ile Ser Ser
 1               5                  10                  15
Gly Val Val Ala Ile Pro Thr Val Ala Ser Ala Cys Gly Met Gly Glu
            20                  25                  30
Val Met Lys Gln Glu Asp Gln Glu His Lys Arg Val Lys Arg Trp Ser
        35                  40                  45
Ala Glu His Pro His His Ala Asn Glu Ser Thr His Leu Trp Ile Ala
    50                  55                  60
Arg Asn Ala Ile Gln Ile Met Ser Arg Asn Gln Asp Lys Thr Val Gln
65                  70                  75                  80
Glu Asn Glu Leu Gln Phe Leu Lys Ile Pro Glu Tyr Lys Glu Leu Phe
                85                  90                  95
Glu Arg Gly Leu Tyr Asp Ala Asp Tyr Leu Asp Glu Phe Asn Asp Gly
            100                 105                 110
Gly Thr Gly Thr Ile Gly Ile Asp Gly Leu Ile Lys Gly Gly Trp Lys
        115                 120                 125
Ser His Phe Tyr Asp Pro Asp Thr Lys Lys Asn Tyr Lys Gly Glu Glu
    130                 135                 140
Glu Pro Thr Ala Leu Ser Gln Gly Asp Lys Tyr Phe Lys Leu Ala Gly
145                 150                 155                 160
Asp Tyr Phe Lys Lys Glu Asp Trp Lys Gln Ala Phe Tyr Tyr Leu Gly
                165                 170                 175
Val Ala Thr His Tyr Phe Thr Asp Ala Thr Gln Pro Met His Ala Ala
            180                 185                 190
Asn Phe Thr Ala Val Asp Met Ser Ala Ile Lys Phe His Ser Ala Phe
        195                 200                 205
Glu Asn Tyr Val Thr Thr Val Gln Thr Pro Phe Glu Val Lys Asp Asp
    210                 215                 220
Lys Gly Thr Tyr Asn Leu Val Asn Ser Asp Asp Pro Lys Gln Trp Ile
225                 230                 235                 240
His Glu Thr Ala Lys Leu Ala Lys Ala Glu Ile Met Asn Ile Thr Ser
                245                 250                 255
Asp Asn Ile Lys Ser Gln Tyr Asn Lys Gly Asn Lys Asp Leu Trp Gln
            260                 265                 270
Gln Glu Val Met Pro Ala Val Gln Arg Ser Leu Glu Lys Ala Gln Arg
        275                 280                 285
Asn Thr Ala Gly Phe Ile His Leu Trp Phe Lys Thr Tyr Val Gly Lys
    290                 295                 300
Thr Ala Ala Glu Asp Ile Glu Thr Thr Gln Val Lys Asp Ser Asn Gly
305                 310                 315                 320
Glu Ala Ile Gln Glu Gln Lys Lys Tyr Tyr Val Val Pro Ser Glu Phe
                325                 330                 335
Leu Asn Arg Gly Leu Thr Phe Glu Val Tyr Ala Ser Asn Asp Tyr Ala
            340                 345                 350
Leu Leu Ser Asn His Val Asp Asp Asn Lys Val His Gly Thr Pro Val
        355                 360                 365
Gln Phe Val Phe Asp Lys Glu Asn Asn Gly Ile Val His Arg Gly Glu
    370                 375                 380
Ser Val Leu Leu Lys Met Thr Gln Ser Asn Tyr Asp Asp Tyr Val Phe
385                 390                 395                 400
Leu Asn Tyr Ser Asn Met Thr Asn Trp Leu His Leu Ala Lys Arg Lys
```

```
                405                 410                 415
Thr Asn Thr Ala Gln Phe Lys Val Tyr Pro Asn Pro Asp Asn Ser Ser
            420                 425                 430
Glu Tyr Phe Leu Tyr Thr Asp Gly Tyr Pro Val Asn Tyr Gln Glu Asn
        435                 440                 445
Gly Asn Gly Lys-Ser Trp Ile Glu Leu Gly Lys Lys Thr Asp Lys Pro
    450                 455                 460
Lys Ala Trp Lys Phe Gln Gln Ala Glu
465                 470
```

<210> 33
<211> 792
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 33

```
atgagagcac tcgtgctggc aggcggtgga gccaagggct cgtttcaagt gggcgtgctg     60
cagcggttca cccccgcaga cttcggtctc gtggtgggat gctcggtcgg agctttaaac    120
gccgcggggt ttgcccacct gggtagccat ggcatcaaag acctctggca agggatcagg    180
agtcgagatg acatcctgtc ccgtgtctgg tggccgtttg gctcagacgg gatcttctcg    240
cagaagcctc ttgaaaagct cgtctccaaa gcatgcacgg gtcctgctcg ggtgccggtc    300
cacgtggcga cggtctgcct tgaacgcggc cttgtccact acgggatctc cggggactct    360
gactttgaga agaaagtgct ggcatcggct gcgatcccag gcgtggtgaa gccagttaag    420
atccatggcg accactacgt cgacggtggt gtcagagaga tctgtccgct gcgtcgagcc    480
atcgacctgg gcgccacgga gatcacagtc atcatgtgcg ctccggaata catcccgacc    540
tggtcgcgta gttcctcgct gttcccgttt gtgaacgtga tgatccggtc tctcgacatc    600
ctgaccgatg agatcctggt caacgacatc gccgagtgcg tggcaaagaa caagatgcca    660
ggtaaacgtc acgtaaagct caccatctac cggccgaaga aagagctcat gggcacgctc    720
gactttgacc ccaaagccat cgccgcaggg atcaaggcag gcaccgaagc ccagccaagg    780
ttctgggagt aa                                                        792
```

<210> 34
<211> 263
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 34

```
Met Arg Ala Leu Val Leu Ala Gly Gly Gly Ala Lys Gly Ser Phe Gln
1               5                   10                  15
Val Gly Val Leu Gln Arg Phe Thr Pro Ala Asp Phe Gly Leu Val Val
            20                  25                  30
Gly Cys Ser Val Gly Ala Leu Asn Ala Ala Gly Phe Ala His Leu Gly
        35                  40                  45
Ser His Gly Ile Lys Asp Leu Trp Gln Gly Ile Arg Ser Arg Asp Asp
    50                  55                  60
Ile Leu Ser Arg Val Trp Trp Pro Phe Gly Ser Asp Gly Ile Phe Ser
65                  70                  75                  80
Gln Lys Pro Leu Glu Lys Leu Val Ser Lys Ala Cys Thr Gly Pro Ala
                85                  90                  95
Arg Val Pro Val His Val Ala Thr Val Cys Leu Glu Arg Gly Leu Val
            100                 105                 110
```

```
His Tyr Gly Ile Ser Gly Asp Ser Asp Phe Glu Lys Lys Val Leu Ala
        115                 120                 125
Ser Ala Ala Ile Pro Gly Val Val Lys Pro Val Lys Ile His Gly Asp
    130                 135                 140
His Tyr Val Asp Gly Gly Val Arg Glu Ile Cys Pro Leu Arg Arg Ala
145                 150                 155                 160
Ile Asp Leu Gly Ala Thr Glu Ile Thr Val Ile Met Cys Ala Pro Glu
                165                 170                 175
Tyr Ile Pro Thr Trp Ser Arg Ser Ser Ser Leu Phe Pro Phe Val Asn
            180                 185                 190
Val Met Ile Arg Ser Leu Asp Ile Leu Thr Asp Glu Ile Leu Val Asn
        195                 200                 205
Asp Ile Ala Glu Cys Val Ala Lys Asn Lys Met Pro Gly Lys Arg His
    210                 215                 220
Val Lys Leu Thr Ile Tyr Arg Pro Lys Lys Glu Leu Met Gly Thr Leu
225                 230                 235                 240
Asp Phe Asp Pro Lys Ala Ile Ala Ala Gly Ile Lys Ala Gly Thr Glu
                245                 250                 255
Ala Gln Pro Arg Phe Trp Glu
            260
```

<210> 35
<211> 1389
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 35

```
atgcccgagc cgcccgccgc atgccgttgc gattgcgcct gcgagcgcga ccagcacctt      60
ttttgcaagg gacccaagcg tatcctcgcg ctcgacggcg gcggcgtgcg cggcgccgtc     120
agcgtcgcat tcctcgaacg gatcgaggcg gtgctcgagg cccggctcgg acgcaaggtg     180
ctgctcggcc actggttcga cctgatcggc ggcacctcga cgggcgccat catcggcggc     240
gcgctggcga tgggattcgc ggccgaggac gtccaaagat tctatcacga gctcgcgccg     300
cgggtgttca ggcatccgct cctgcgcatc ggtctcctgc gcccgttccg cgcgaaattc     360
gacgcccgcc tgctgcgcga ggagatccac cgcatcatcg gcgacagcac gctcggcgac     420
aaagcgctga tgaccgggtt cgcgctcgtc gccaagcgga tggacaccgg cagcacctgg     480
atcctcgcca acaacaagcg cagcaaatac tgggaagggc gggacggcgt cgtcggcaac     540
aaggattatc tcctcggcag cctcattcgc gcgagcacgg cggcgccgct gtatttcgac     600
cccgaggagg tcgtgatcgc ggaggcccgc aaggacatcg agggcatcag gggcctgttc     660
gtcgacggcg gcgtcacgcc gcacaacaat ccttcgctcg cgatgctgct gctggcgctg     720
ctcgacgcct accggctgcg ctgggaaacg ggaccggaca agctcacggt cgtctcgatc     780
ggcactggaa cgcatcgcga ccgcgtcgtt cccgacacgc tcggcatggg caagaacgcg     840
aagatcgcgc tgcgcgccat gagctcgctg atgaacgacg tgcacgagct cgcgctcacg     900
cagatgcagt acctcggtga gacgctcacc ccgtggcgca tcaacgacga gctcggcgac     960
atgcggaccg agcggccgcc gcaaggcaag ctcttccgct tcctccgcta cgacgtccgg    1020
ctggagctcg attggatcaa cgaggacgag gagcgccggc gcaagatcaa gaacaaattc    1080
aagcgcgagc tgaccgagac cgacatgatc cgcctgcgca gcctcgacga tccgacgacc    1140
atcccggacc tctacatgct tgcccaggtc gcggccgagg agcaggtcaa ggcggagcac    1200
tggctcggcg acgtgccgga gtggagcgaa ggcgcgcgcc cgtgtgcgcc gcgccggcac    1260
ctgccgccga cgccgccggg ccgctccgag gattcggcgc gcttccgggc cgagaaggcc    1320
gtcggcgagt ggctcagttt tgcgcgcgcg aacatcacgc gcctcatgtc gcggaagccg    1380
ccgggttga                                                            1389
```

<210> 36
<211> 462

```
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 36
Met Pro Glu Pro Pro Ala Ala Cys Arg Cys Asp Cys Ala Cys Glu Arg
1               5                   10                  15
Asp Gln His Leu Phe Cys Lys Gly Pro Lys Arg Ile Leu Ala Leu Asp
            20                  25                  30
Gly Gly Gly Val Arg Gly Ala Val Ser Val Ala Phe Leu Glu Arg Ile
        35                  40                  45
Glu Ala Val Leu Glu Ala Arg Leu Gly Arg Lys Val Leu Leu Gly His
    50                  55                  60
Trp Phe Asp Leu Ile Gly Gly Thr Ser Thr Gly Ala Ile Ile Gly Gly
65                  70                  75                  80
Ala Leu Ala Met Gly Phe Ala Ala Glu Asp Val Gln Arg Phe Tyr His
                85                  90                  95
Glu Leu Ala Pro Arg Val Phe Arg His Pro Leu Leu Arg Ile Gly Leu
            100                 105                 110
Leu Arg Pro Phe Arg Ala Lys Phe Asp Ala Arg Leu Leu Arg Glu Glu
        115                 120                 125
Ile His Arg Ile Ile Gly Asp Ser Thr Leu Gly Asp Lys Ala Leu Met
    130                 135                 140
Thr Gly Phe Ala Leu Val Ala Lys Arg Met Asp Thr Gly Ser Thr Trp
145                 150                 155                 160
Ile Leu Ala Asn Asn Lys Arg Ser Lys Tyr Trp Glu Gly Arg Asp Gly
                165                 170                 175
Val Val Gly Asn Lys Asp Tyr Leu Leu Gly Ser Leu Ile Arg Ala Ser
            180                 185                 190
Thr Ala Ala Pro Leu Tyr Phe Asp Pro Glu Glu Val Val Ile Ala Glu
        195                 200                 205
Ala Arg Lys Asp Ile Glu Gly Ile Arg Gly Leu Phe Val Asp Gly Gly
    210                 215                 220
Val Thr Pro His Asn Asn Pro Ser Leu Ala Met Leu Leu Leu Ala Leu
225                 230                 235                 240
Leu Asp Ala Tyr Arg Leu Arg Trp Glu Thr Gly Pro Asp Lys Leu Thr
                245                 250                 255
Val Val Ser Ile Gly Thr Gly Thr His Arg Asp Arg Val Val Pro Asp
            260                 265                 270
Thr Leu Gly Met Gly Lys Asn Ala Lys Ile Ala Leu Arg Ala Met Ser
        275                 280                 285
Ser Leu Met Asn Asp Val His Glu Leu Ala Leu Thr Gln Met Gln Tyr
    290                 295                 300
Leu Gly Glu Thr Leu Thr Pro Trp Arg Ile Asn Asp Glu Leu Gly Asp
305                 310                 315                 320
Met Arg Thr Glu Arg Pro Pro Gln Gly Lys Leu Phe Arg Phe Leu Arg
                325                 330                 335
Tyr Asp Val Arg Leu Glu Leu Asp Trp Ile Asn Glu Asp Glu Glu Arg
            340                 345                 350
Arg Arg Lys Ile Lys Asn Lys Phe Lys Arg Glu Leu Thr Glu Thr Asp
        355                 360                 365
Met Ile Arg Leu Arg Ser Leu Asp Asp Pro Thr Thr Ile Pro Asp Leu
    370                 375                 380
Tyr Met Leu Ala Gln Val Ala Ala Glu Glu Gln Val Lys Ala Glu His
385                 390                 395                 400
```

```
Trp Leu Gly Asp Val Pro Glu Trp Ser Glu Gly Ala Arg Pro Cys Ala
                405                 410                 415
Pro Arg Arg His Leu Pro Pro Thr Pro Pro Gly Arg Ser Glu Asp Ser
            420                 425                 430
Ala Arg Phe Arg Ala Glu Lys Ala Val Gly Glu Trp Leu Ser Phe Ala
        435                 440                 445
Arg Ala Asn Ile Thr Arg Leu Met Ser Arg Lys Pro Pro Gly
    450                 455                 460

<210> 37
<211> 1329
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 37
atgagaaatt tcagcaaggg attgaccagt attttgctta gcatagcgac atccaccagt      60
gcgatggcct ttacccagat cggggccggc ggagcgattc cgatgggcca tgagtggcta     120
acccgccgct cggcgctgga actgctgaat gccgacaatc tggtcggcaa tgacccggcc     180
gacccacgct tgggctggag cgaaggtctc gccaacaatc tcgatctctc gaatgcccag     240
aacgaagtgc agcgcatcaa gagcattacc aagagccacg ccctgtatga gccgcgttac     300
gatgacgttt tcgccgccat cgtcggcgag cgctgggttg ataccgccgg tttcaacgtg     360
gccaaggcca ccgtcggcaa gatcgattgc ttcagcgccg tcgcgcaaga gcccgccgat     420
gtgcaacaag accatttcat gcgccgttat gacgacgtgg gtggacaagg gggcgtgaac     480
gctgcccgcc gcgcgcagca gcgctttatc aatcacttcg tcaacgcagc catggccgaa     540
gagaagagca tcaaggcatg ggatggcggc ggttattctt cgctggaaaa agtcagccac     600
aactacttct tgtttggccg cgccgttcat ttgttccagg attctttcag ccccgaacac     660
accgtgcgcc tgcctgaaga caattacgtc aaagtccgtc aggtcaaggc gtatctctgc     720
tctgaaggtg ccgaacagca tacgcacaac acgcaagatg ccatcaactt caccagcggc     780
gatgtcatct ggaaacagaa cacccgtctg gatgcaggct ggagcaccta caaggccagc     840
aacatgaagc cggtggcatt ggttgccctc gaagccagca aagatttgtg ggccgccttt     900
attcgcacca tggccgtttc ccgcgaggag cgtcgcgccg tcgccgaaca ggaagcgcag     960
gctctcgtca atcactggtt gtcgttcgac gaacaggaaa tgctgaactg gtacgaagaa    1020
gaagagcacc gcgatcatac gtacgtcaag gaacccggcc agagcggccc aggttcgtcg    1080
ttattcgatt gcatggttgg tctgggtgtg gcctcgggca gtcaggcgca acgggtggcg    1140
gaactcgatc agcaacgccg ccaatgtttg ttcaacgtca aggccgctac tggctatggc    1200
gatctgaatg atccacacat ggatattccg tacaactggc aatgggtgtc gtcgacgcaa    1260
tggaaaatcc ctgcggccga ctggaaaatc ccgcagctgc ccgccgattc agggaaatca    1320
gtcgtcatc                                                            1329

<210> 38
<211> 443
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(23)

<400> 38
Met Arg Asn Phe Ser Lys Gly Leu Thr Ser Ile Leu Leu Ser Ile Ala
 1               5                  10                  15
Thr Ser Thr Ser Ala Met Ala Phe Thr Gln Ile Gly Ala Gly Gly Ala
```

```
            20                  25                  30
Ile Pro Met Gly His Glu Trp Leu Thr Arg Arg Ser Ala Leu Glu Leu
        35                  40                  45
Leu Asn Ala Asp Asn Leu Val Gly Asn Asp Pro Ala Asp Pro Arg Leu
    50                  55                  60
Gly Trp Ser Glu Gly Leu Ala Asn Asn Leu Asp Leu Ser Asn Ala Gln
65                  70                  75                  80
Asn Glu Val Gln Arg Ile Lys Ser Ile Thr Lys Ser His Ala Leu Tyr
                85                  90                  95
Glu Pro Arg Tyr Asp Asp Val Phe Ala Ala Ile Val Gly Glu Arg Trp
            100                 105                 110
Val Asp Thr Ala Gly Phe Asn Val Ala Lys Ala Thr Val Gly Lys Ile
        115                 120                 125
Asp Cys Phe Ser Ala Val Ala Gln Glu Pro Ala Asp Val Gln Gln Asp
    130                 135                 140
His Phe Met Arg Arg Tyr Asp Asp Val Gly Gly Gln Gly Gly Val Asn
145                 150                 155                 160
Ala Ala Arg Arg Ala Gln Gln Arg Phe Ile Asn His Phe Val Asn Ala
                165                 170                 175
Ala Met Ala Glu Glu Lys Ser Ile Lys Ala Trp Asp Gly Gly Gly Tyr
            180                 185                 190
Ser Ser Leu Glu Lys Val Ser His Asn Tyr Phe Leu Phe Gly Arg Ala
        195                 200                 205
Val His Leu Phe Gln Asp Ser Phe Ser Pro Glu His Thr Val Arg Leu
    210                 215                 220
Pro Glu Asp Asn Tyr Val Lys Val Arg Gln Val Lys Ala Tyr Leu Cys
225                 230                 235                 240
Ser Glu Gly Ala Glu Gln His Thr His Asn Thr Gln Asp Ala Ile Asn
                245                 250                 255
Phe Thr Ser Gly Asp Val Ile Trp Lys Gln Asn Thr Arg Leu Asp Ala
            260                 265                 270
Gly Trp Ser Thr Tyr Lys Ala Ser Asn Met Lys Pro Val Ala Leu Val
        275                 280                 285
Ala Leu Glu Ala Ser Lys Asp Leu Trp Ala Ala Phe Ile Arg Thr Met
    290                 295                 300
Ala Val Ser Arg Glu Glu Arg Arg Ala Val Ala Glu Gln Glu Ala Gln
305                 310                 315                 320
Ala Leu Val Asn His Trp Leu Ser Phe Asp Glu Gln Glu Met Leu Asn
                325                 330                 335
Trp Tyr Glu Glu Glu Glu His Arg Asp His Thr Tyr Val Lys Glu Pro
            340                 345                 350
Gly Gln Ser Gly Pro Gly Ser Ser Leu Phe Asp Cys Met Val Gly Leu
        355                 360                 365
Gly Val Ala Ser Gly Ser Gln Ala Gln Arg Val Ala Glu Leu Asp Gln
    370                 375                 380
Gln Arg Arg Gln Cys Leu Phe Asn Val Lys Ala Ala Thr Gly Tyr Gly
385                 390                 395                 400
Asp Leu Asn Asp Pro His Met Asp Ile Pro Tyr Asn Trp Gln Trp Val
                405                 410                 415
Ser Ser Thr Gln Trp Lys Ile Pro Ala Ala Asp Trp Lys Ile Pro Gln
            420                 425                 430
Leu Pro Ala Asp Ser Gly Lys Ser Val Val Ile
        435                 440
```

<210> 39
<211> 1335
<212> DNA

<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 39
atggccaacc ccatcgtcat catccacggc tggagcgacg acttcggctc gttccgcaag 60
ctgcgcgact tcctctccac caacctcggc gttccggcga agatcctcaa gctcggcgac 120
tggatctcgc tcgacgacga cgtcggctac gccgacatcg cgatggcgct ggaacgcgcg 180
tggaaggcgg agaaactgcc gaccgcgccg cgttcggtcg acgtcgtcgt gcacagcacc 240
ggcgcgctgg tggtgcgcga atggatgacg cgctaccacg cgcccgaaac cgtgccgatc 300
cagcgcttcc tgcacctggc gccggccaac ttcggctcgc acctcgcgca caagggccgc 360
tcgttcatcg gccgcgcggt gaagggctgg aagaccggct tcgaaaccgg cacccgcatc 420
ctgcgcgggc tggaactcgc ctcgccctac tcgcgcgcgc tggccgagcg cgacctgttc 480
gtggcgccgt cgaagcgctg gtacggcgcc ggccgcatcc tcgccaccgt gctggtcggc 540
aacagcggct actccggcat ccaggccatc gccaacgagg acggctccga cggcaccgtg 600
cgcatcggca ccgccaacct gcaggcggcg cttgcgaagg tggtgttccc gcccggcccg 660
gtcgcgccgg tggtgcagtt ccgcaacatc gcgggcgcca ccgcgttcgc catcgtcgac 720
ggcgacaacc attccgacat caccatgaag gacaagccgt cgaagaccgg catccgcgag 780
gaactgatcc tcggcgcgct gaaggtgcgc gacgccgact tccccgagaa cgccgacggc 840
gcgttcccgt ggcaggcgaa gctcgacgcg aaggccggtg cggccaaggt gtcttcgccc 900
gggcgccaga acaccgtggt gcacctcacc gacagcttcg gcgacgacgt cgtcgatttc 960
ttcttcgagt tctggcgcag cgaacgcagc gacaaggtgt tcgagcagcg cttctacaag 1020
gacgtcatcg acgacgtgca cgtgtacgac ggcaacggcg cgtggcgctc gctcaacctc 1080
gacctcgaca agttcgaggc gctgcgcaag gacccgaagc tcggcttcga gaaactgctg 1140
gtcagcgtgt tcgcctcgcc cgcgaagaag ggcgacgcca aggtcggcta cagcaccgcc 1200
accggccgcg acatcggcgc ctggcacgtc gaaggccgtg acttcgccaa ggccttcacg 1260
ccgcaccgca ccctgttcgt cgacatcgag atcccacgca tcgtcgacga cgcggtgttc 1320
cggttccggg aatag 1335

<210> 40
<211> 444
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 40
Met Ala Asn Pro Ile Val Ile Ile His Gly Trp Ser Asp Asp Phe Gly
1 5 10 15
Ser Phe Arg Lys Leu Arg Asp Phe Leu Ser Thr Asn Leu Gly Val Pro
20 25 30
Ala Lys Ile Leu Lys Leu Gly Asp Trp Ile Ser Leu Asp Asp Asp Val
35 40 45
Gly Tyr Ala Asp Ile Ala Met Ala Leu Glu Arg Ala Trp Lys Ala Glu
50 55 60
Lys Leu Pro Thr Ala Pro Arg Ser Val Asp Val Val Val His Ser Thr
65 70 75 80
Gly Ala Leu Val Val Arg Glu Trp Met Thr Arg Tyr His Ala Pro Glu
85 90 95
Thr Val Pro Ile Gln Arg Phe Leu His Leu Ala Pro Ala Asn Phe Gly
100 105 110
Ser His Leu Ala His Lys Gly Arg Ser Phe Ile Gly Arg Ala Val Lys
115 120 125
Gly Trp Lys Thr Gly Phe Glu Thr Gly Thr Arg Ile Leu Arg Gly Leu
130 135 140

```
Glu Leu Ala Ser Pro Tyr Ser Arg Ala Leu Ala Glu Arg Asp Leu Phe
145                 150                 155                 160
Val Ala Pro Ser Lys Arg Trp Tyr Gly Ala Gly Arg Ile Leu Ala Thr
                165                 170                 175
Val Leu Val Gly Asn Ser Gly Tyr Ser Gly Ile Gln Ala Ile Ala Asn
            180                 185                 190
Glu Asp Gly Ser Asp Gly Thr Val Arg Ile Gly Thr Ala Asn Leu Gln
        195                 200                 205
Ala Ala Leu Ala Lys Val Val Phe Pro Pro Gly Pro Val Ala Pro Val
    210                 215                 220
Val Gln Phe Arg Asn Ile Ala Gly Ala Thr Ala Phe Ala Ile Val Asp
225                 230                 235                 240
Gly Asp Asn His Ser Asp Ile Thr Met Lys Asp Lys Pro Ser Lys Thr
                245                 250                 255
Gly Ile Arg Glu Glu Leu Ile Leu Gly Ala Leu Lys Val Arg Asp Ala
            260                 265                 270
Asp Phe Pro Glu Asn Ala Asp Gly Ala Phe Pro Trp Gln Ala Lys Leu
        275                 280                 285
Asp Ala Lys Ala Gly Ala Ala Lys Val Ser Ser Pro Gly Arg Gln Asn
    290                 295                 300
Thr Val Val His Leu Thr Asp Ser Phe Gly Asp Asp Val Val Asp Phe
305                 310                 315                 320
Phe Phe Glu Phe Trp Arg Ser Glu Arg Ser Asp Lys Val Phe Glu Gln
                325                 330                 335
Arg Phe Tyr Lys Asp Val Ile Asp Asp Val His Val Tyr Asp Gly Asn
            340                 345                 350
Gly Ala Trp Arg Ser Leu Asn Leu Asp Leu Asp Lys Phe Glu Ala Leu
        355                 360                 365
Arg Lys Asp Pro Lys Leu Gly Phe Glu Lys Leu Leu Val Ser Val Phe
    370                 375                 380
Ala Ser Pro Ala Lys Lys Gly Asp Ala Lys Val Gly Tyr Ser Thr Ala
385                 390                 395                 400
Thr Gly Arg Asp Ile Gly Ala Trp His Val Glu Gly Arg Asp Phe Ala
                405                 410                 415
Lys Ala Phe Thr Pro His Arg Thr Leu Phe Val Asp Ile Glu Ile Pro
            420                 425                 430
Arg Ile Val Asp Asp Ala Val Phe Arg Phe Arg Glu
        435                 440
```

<210> 41
<211> 1419
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 41

```
atgacgctcc gatcaacgga ctatgcgctg ctggcgcagg agagctacca cgacagccag      60
gtggacgccg acgtcaagct ggatggcgtg gcgtataaag tcttcgccac caccagcgac     120
gggctcaccg gattccaggc cacggcctac cagcgccagg acaccggcga ggtagtgatt     180
gcgtaccgcg gcacggagtt tgatcgcgag cccgtccgcg acggcggcgt cgatgcgggc     240
atggtgctgc tcggtgtcaa cgcacaggca ccagcgtcgg aagtgttcac ccggcaagtg     300
atcgagaagg cgaaacacga agccgagctc aacgaccgcg aaccgcagat caccgtcacc     360
ggccattccc tcggcggcac cctcgccgag atcaacgccg cgaagtacgg cctccatggc     420
gaaaccttca acgcctacgg cgcagccagc ctcaagggta ttccggaggg cggcgatacc     480
gtcatcgacc acgtccgtgc cggcgatctc gtcagcgcgg ccagcccccca ctacgggcag     540
```

gtacgcgtct acgcggcgca gcaggacatc gatacgctgc aacacgccgg ttaccgcgat 600
gacagcggca tcctcagctt gcgcaacccg atcaaggcca cggatttcga tgcccatgcc 660
atcgataact tcgtgcccaa cagcaagctg ctcggtcagt cgatcatcgc gccggaaaac 720
gtggcgcgtt acgatgccca caaaggcatg gtcgaccgtt accgcgatga cgtggccgat 780
atccgcaagg gcatctcggc gccctgggaa atccccaagg ccatcggcga gctgaaggac 840
accctggagc acgaagcctt cgaactcgcc ggcaagggca ttctcgcggt ggagcacggc 900
ttcgaacatc tcaaggagga gatcggcgaa ggcatccacg ccgtggagga gaaagcttcc 960
agcgcgtggc ataccctcac ccatcccaag gaatggttcg agcacgataa acccaaggtg 1020
accctggacc acccggacca ccccgaccat gccctgttca agcaggcgca gggcgcggtg 1080
cacacagtcg atgcctcgca cggccgcacc cctgacaaga ccagcgacca gatcgccggc 1140
tcgctggtgg tatcggcacg ccgtgacggc cttgagcggg tagaccgcgc tgtactcagc 1200
gatgacgcca accgcctgta cggtgtgcag ggtgcggtgg actcgccgct gaagcaggtc 1260
accgaagtga acaccgccac cgccgcgcag acatcgctcc agcagagcag cgtggcctgg 1320
cagcaacagg cagaaatcgc gcgtcagaac caggcggcaa gccaggctca gcgcatggac 1380
cagcaggtgc cgccgcaggc acccgcgcac ggcatgtaa 1419

<210> 42
<211> 472
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 42
Met Thr Leu Arg Ser Thr Asp Tyr Ala Leu Leu Ala Gln Glu Ser Tyr
1 5 10 15
His Asp Ser Gln Val Asp Ala Asp Val Lys Leu Asp Gly Val Ala Tyr
20 25 30
Lys Val Phe Ala Thr Thr Ser Asp Gly Leu Thr Gly Phe Gln Ala Thr
35 40 45
Ala Tyr Gln Arg Gln Asp Thr Gly Glu Val Val Ile Ala Tyr Arg Gly
50 55 60
Thr Glu Phe Asp Arg Glu Pro Val Arg Asp Gly Gly Val Asp Ala Gly
65 70 75 80
Met Val Leu Leu Gly Val Asn Ala Gln Ala Pro Ala Ser Glu Val Phe
85 90 95
Thr Arg Gln Val Ile Glu Lys Ala Lys His Glu Ala Glu Leu Asn Asp
100 105 110
Arg Glu Pro Gln Ile Thr Val Thr Gly His Ser Leu Gly Gly Thr Leu
115 120 125
Ala Glu Ile Asn Ala Ala Lys Tyr Gly Leu His Gly Glu Thr Phe Asn
130 135 140
Ala Tyr Gly Ala Ala Ser Leu Lys Gly Ile Pro Glu Gly Gly Asp Thr
145 150 155 160
Val Ile Asp His Val Arg Ala Gly Asp Leu Val Ser Ala Ala Ser Pro
165 170 175
His Tyr Gly Gln Val Arg Val Tyr Ala Ala Gln Gln Asp Ile Asp Thr
180 185 190
Leu Gln His Ala Gly Tyr Arg Asp Asp Ser Gly Ile Leu Ser Leu Arg
195 200 205
Asn Pro Ile Lys Ala Thr Asp Phe Asp Ala His Ala Ile Asp Asn Phe
210 215 220
Val Pro Asn Ser Lys Leu Leu Gly Gln Ser Ile Ile Ala Pro Glu Asn
225 230 235 240
Val Ala Arg Tyr Asp Ala His Lys Gly Met Val Asp Arg Tyr Arg Asp
245 250 255

Asp Val Ala Asp Ile Arg Lys Gly Ile Ser Ala Pro Trp Glu Ile Pro
260 265 270
Lys Ala Ile Gly Glu Leu Lys Asp Thr Leu Glu His Glu Ala Phe Glu
275 280 285
Leu Ala Gly Lys Gly Ile Leu Ala Val Glu His Gly Phe Glu His Leu
290 295 300
Lys Glu Glu Ile Gly Glu Gly Ile His Ala Val Glu Glu Lys Ala Ser
305 310 315 320
Ser Ala Trp His Thr Leu Thr His Pro Lys Glu Trp Phe Glu His Asp
325 330 335
Lys Pro Lys Val Thr Leu Asp His Pro Asp His Pro Asp His Ala Leu
340 345 350
Phe Lys Gln Ala Gln Gly Ala Val His Thr Val Asp Ala Ser His Gly
355 360 365
Arg Thr Pro Asp Lys Thr Ser Asp Gln Ile Ala Gly Ser Leu Val Val
370 375 380
Ser Ala Arg Arg Asp Gly Leu Glu Arg Val Asp Arg Ala Val Leu Ser
385 390 395 400
Asp Asp Ala Asn Arg Leu Tyr Gly Val Gln Gly Ala Val Asp Ser Pro
405 410 415
Leu Lys Gln Val Thr Glu Val Asn Thr Ala Thr Ala Ala Gln Thr Ser
420 425 430
Leu Gln Gln Ser Ser Val Ala Trp Gln Gln Gln Ala Glu Ile Ala Arg
435 440 445
Gln Asn Gln Ala Ala Ser Gln Ala Gln Arg Met Asp Gln Gln Val Pro
450 455 460
Pro Gln Ala Pro Ala His Gly Met
465 470

<210> 43
<211> 1287
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 43
atgtcgatta ccgtttaccg gaagccctcc ggcgggtttg gagcgatagt tcctcaagcg 60
aaaattgaga accttgtttt cgagggcggc ggaccaaagg gcctggtcta tgtcggcgcg 120
gtcgaggttc tcggtgaaag gggactgctg gaagggatcg caaatgtcgg cggcgcttca 180
gcaggcgcca tgaccgctct agccgtcggt ctgggactga gccccaggga aattcgcgcg 240
gtcgtcttta accagaacat tgcggacctc accgatatcg agaagaccgt cgagccgtcc 300
tccgggatca caggcatgtt caagagcgtg ttcaagaagg gttggcaggc ggtgcgcaac 360
gtaaccggca cctctgacga gcgcgggcgc gggctctatc gcggcgagaa gttgcgagcc 420
tggatcagag acctgattgc acagcgagtc gaggcagggc gctcagaggt gctgagccga 480
gccgacgccg acgggcggaa cttctatgag aaagccgccg caaagaaggg cgccctgaca 540
tttgccgaac ttgatcgggt ggcgcaaatg gcgccgggcc tgcggcttcg ccgcctggcc 600
ttcaccggaa ccaacttcac gtcgaagaag ctcgaagtgt tcagtctgca cgagaccccg 660
gacatgccga tcgacgtcgc ggtacgcatc tcggcatcgt tgccatggtt tttcaaatcc 720
gtgaaatgga acggctccga atacatagat ggcggatgcc tgtcgaactt cccaatgccg 780
atattcgacg tcgatcccta tcgtggcgac gcatcgtcga agatccggct cggcatcttc 840
ggccagaacc tcgcgacgct cggcttcaag gtcgacagcg aggaggagat ccgcgacatc 900
ctctggcgta gccccgagag cacgagcgac ggctttttcc aaggcatcct gtcaagcgtg 960
aaagcctcgg cagaacactg ggtcgtcggc atcgatgtcg agggcgccac ccgcgcgtcg 1020
aacgtggccg ttcacggcaa gtatgctcag cgaacgatcc agataccgga cctcggatat 1080
agcacgttca agttcgatct ctcagacgcg gacaaggagc gcatggccga ggccggcgca 1140

```
aaggccacgc gggaatggct ggcgctgtac ttcgacgacg ccggaataga ggtcgaattt     1200
tctgatccga acgaattgcg cggccagttg tccgacgccg cattcgcaga cctcgaggat     1260
tcgtttcgag ccttgatcgc ggcctag                                         1287

<210> 44
<211> 428
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 44
Met Ser Ile Thr Val Tyr Arg Lys Pro Ser Gly Gly Phe Gly Ala Ile
1               5                   10                  15
Val Pro Gln Ala Lys Ile Glu Asn Leu Val Phe Glu Gly Gly Gly Pro
            20                  25                  30
Lys Gly Leu Val Tyr Val Gly Ala Val Glu Val Leu Gly Glu Arg Gly
        35                  40                  45
Leu Leu Glu Gly Ile Ala Asn Val Gly Gly Ala Ser Ala Gly Ala Met
    50                  55                  60
Thr Ala Leu Ala Val Gly Leu Gly Leu Ser Pro Arg Glu Ile Arg Ala
65                  70                  75                  80
Val Val Phe Asn Gln Asn Ile Ala Asp Leu Thr Asp Ile Glu Lys Thr
                85                  90                  95
Val Glu Pro Ser Ser Gly Ile Thr Gly Met Phe Lys Ser Val Phe Lys
            100                 105                 110
Lys Gly Trp Gln Ala Val Arg Asn Val Thr Gly Thr Ser Asp Glu Arg
        115                 120                 125
Gly Arg Gly Leu Tyr Arg Gly Glu Lys Leu Arg Ala Trp Ile Arg Asp
    130                 135                 140
Leu Ile Ala Gln Arg Val Glu Ala Gly Arg Ser Glu Val Leu Ser Arg
145                 150                 155                 160
Ala Asp Ala Asp Gly Arg Asn Phe Tyr Glu Lys Ala Ala Ala Lys Lys
                165                 170                 175
Gly Ala Leu Thr Phe Ala Glu Leu Asp Arg Val Ala Gln Met Ala Pro
            180                 185                 190
Gly Leu Arg Leu Arg Arg Leu Ala Phe Thr Gly Thr Asn Phe Thr Ser
        195                 200                 205
Lys Lys Leu Glu Val Phe Ser Leu His Glu Thr Pro Asp Met Pro Ile
    210                 215                 220
Asp Val Ala Val Arg Ile Ser Ala Ser Leu Pro Trp Phe Phe Lys Ser
225                 230                 235                 240
Val Lys Trp Asn Gly Ser Glu Tyr Ile Asp Gly Gly Cys Leu Ser Asn
                245                 250                 255
Phe Pro Met Pro Ile Phe Asp Val Asp Pro Tyr Arg Gly Asp Ala Ser
            260                 265                 270
Ser Lys Ile Arg Leu Gly Ile Phe Gly Gln Asn Leu Ala Thr Leu Gly
        275                 280                 285
Phe Lys Val Asp Ser Glu Glu Glu Ile Arg Asp Ile Leu Trp Arg Ser
    290                 295                 300
Pro Glu Ser Thr Ser Asp Gly Phe Phe Gln Gly Ile Leu Ser Ser Val
305                 310                 315                 320
Lys Ala Ser Ala Glu His Trp Val Val Gly Ile Asp Val Glu Gly Ala
                325                 330                 335
Thr Arg Ala Ser Asn Val Ala Val His Gly Lys Tyr Ala Gln Arg Thr
            340                 345                 350
```

Ile Gln Ile Pro Asp Leu Gly Tyr Ser Thr Phe Lys Phe Asp Leu Ser
355 360 365
Asp Ala Asp Lys Glu Arg Met Ala Glu Ala Gly Ala Lys Ala Thr Arg
370 375 380
Glu Trp Leu Ala Leu Tyr Phe Asp Asp Ala Gly Ile Glu Val Glu Phe
385 390 395 400
Ser Asp Pro Asn Glu Leu Arg Gly Gln Leu Ser Asp Ala Ala Phe Ala
405 410 415
Asp Leu Glu Asp Ser Phe Arg Ala Leu Ile Ala Ala
420 425

<210> 45
<211> 1038
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 45
atgacaaccc aatttagaaa cttgatattt gaaggcggcg gtgtaaaagg tgttgcttac 60
attggcgcca tgcagattct cgaaaatcgt ggcgtgttgc aagatattca ccgagtcgga 120
gggtgcagtg cgggtgcgat taatgcgctg atttttgcgc tgggttacac ggttcgtgag 180
caaaaagaga tcttacaagc caccgatttt aaccagttta tggataactc ttggggtgtt 240
attcgtgata ttcgcaggct tgctcgagac tttggctgga ataagggtga tttctttagt 300
agctggatag gtgatttgat tcatcgtcgt ttggggaatc gccgagcgac gttcaaagat 360
ctgcaaaatg ccaagcttcc tgatctttat gtcatcggta ctaatctgtc tacagggttt 420
gcagaggttt tttctgccga aagacacccc gatatggagc tggcgacagc ggtgcgtatc 480
tccatgtcga taccgctgtt ctttgcagcc gtgcgtcacg gtgatcgaca agatgtgtat 540
gtcgatgggg gtgttcaact taactatccg attaaactgt ttgatcggga gcgttacatt 600
gatctggcca aagatcccgg tgctgttcgg cgaacgggtt attacaacaa agaaaacgct 660
cgctttcagc ttgagcggcc cggtcatagc cctatgtttt acaatcgcca gaccttgggt 720
ttgcgtcttg atagtcgcga gcagataggg ctctttcgtt atgacgaacc cctcaagggc 780
aaacccatta agtccttcac tgactacgct cgacaacttt tcggtgcgtt gatgaatgca 840
caggaaaaga ttcatctaca tggcgatgat tggcaacgca cggtctatat cgatacattg 900
gatgtgggta cgacggactt caatctttct gatgcaacta agcaagcact gattgagcaa 960
ggaattaacg gcaccgaaaa ttatttcgag tggtttgata atccgttaga gaagcccgtg 1020
aatagagtgg agtcatag 1038

<210> 46
<211> 345
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 46
Met Thr Thr Gln Phe Arg Asn Leu Ile Phe Glu Gly Gly Gly Val Lys
1 5 10 15
Gly Val Ala Tyr Ile Gly Ala Met Gln Ile Leu Glu Asn Arg Gly Val
20 25 30
Leu Gln Asp Ile His Arg Val Gly Gly Cys Ser Ala Gly Ala Ile Asn
35 40 45
Ala Leu Ile Phe Ala Leu Gly Tyr Thr Val Arg Glu Gln Lys Glu Ile
50 55 60
Leu Gln Ala Thr Asp Phe Asn Gln Phe Met Asp Asn Ser Trp Gly Val

```
65                  70                  75                  80
Ile Arg Asp Ile Arg Arg Leu Ala Arg Asp Phe Gly Trp Asn Lys Gly
                85                  90                  95
Asp Phe Phe Ser Ser Trp Ile Gly Asp Leu Ile His Arg Arg Leu Gly
            100                 105                 110
Asn Arg Arg Ala-Thr Phe Lys Asp Leu Gln Asn Ala Lys Leu Pro Asp
        115                 120                 125
Leu Tyr Val Ile Gly Thr Asn Leu Ser Thr Gly Phe Ala Glu Val Phe
    130                 135                 140
Ser Ala Glu Arg His Pro Asp Met Glu Leu Ala Thr Ala Val Arg Ile
145                 150                 155                 160
Ser Met Ser Ile Pro Leu Phe Phe Ala Ala Val Arg His Gly Asp Arg
                165                 170                 175
Gln Asp Val Tyr Val Asp Gly Gly Val Gln Leu Asn Tyr Pro Ile Lys
            180                 185                 190
Leu Phe Asp Arg Glu Arg Tyr Ile Asp Leu Ala Lys Asp Pro Gly Ala
        195                 200                 205
Val Arg Arg Thr Gly Tyr Tyr Asn Lys Glu Asn Ala Arg Phe Gln Leu
    210                 215                 220
Glu Arg Pro Gly His Ser Pro Tyr Val Tyr Asn Arg Gln Thr Leu Gly
225                 230                 235                 240
Leu Arg Leu Asp Ser Arg Glu Gln Ile Gly Leu Phe Arg Tyr Asp Glu
                245                 250                 255
Pro Leu Lys Gly Lys Pro Ile Lys Ser Phe Thr Asp Tyr Ala Arg Gln
            260                 265                 270
Leu Phe Gly Ala Leu Met Asn Ala Gln Glu Lys Ile His Leu His Gly
        275                 280                 285
Asp Asp Trp Gln Arg Thr Val Tyr Ile Asp Thr Leu Asp Val Gly Thr
    290                 295                 300
Thr Asp Phe Asn Leu Ser Asp Ala Thr Lys Gln Ala Leu Ile Glu Gln
305                 310                 315                 320
Gly Ile Asn Gly Thr Glu Asn Tyr Phe Glu Trp Phe Asp Asn Pro Leu
                325                 330                 335
Glu Lys Pro Val Asn Arg Val Glu Ser
            340                 345
```

<210> 47
<211> 1476
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 47

```
atgtcaacaa aagtagtatt tgtacatgga tggagcgtta ccaacctaaa tacatatggc      60
gaacttccgt tgagattaaa ggccgaagca ataagcagga acctgaacat cgaagtaaat     120
gaaattttcc tgggccgtta tatcagcttt aatgataaca ttacattaga tgacgtttcg     180
cgggctttta atacggccat tagcgaacag ttagacaata cagacaggtt tatatgtatt     240
acacattcta ccggagggcc ggttattcgc gaatggttaa ataaatacta ttataatgaa     300
cgtccaccac taagtcattt aataatgctt gcaccggcca attttggttc ggcattggct     360
cgtttaggga aaagtaaatt aagccgtatt aaaagttggt ttgaaggtgt agaaccaggg     420
cagaaaattt tagactggct ggagtgtgga agcaaccaat cgtggttact aaataaagac     480
tggatcgaca atggcaattt tcagattggc gctgataagt atttcccgtt tgttatcatt     540
ggccagtcga ttgatcgtaa actttacgat catcttaact catataccgg cgagcttggg     600
tccgatggtg tagttcgcac ctcaggagct aatcttaatt cgcggtatat taagcttgtt     660
caggacagaa atacaatagc taatggaaat atttccagta cattacgaat tgccgaatat     720
```

```
agagaagctt gtgcaacgcc catacgggta gttagaggta aatcgcattc gggcgatgaa      780
atgggtatca tgaaaagtgt taaaaaagaa attactgatg ccggaagcaa ggaaacaata      840
aatgccatat tcgagtgtat tgaagttaca aacaacgaac aatatcaatc cttaattact      900
aaatttgata acgaaacagc acaggtacaa aaggatgagc tgattgaaac ggaaacagaa      960
ttatttttaa tgcaccgtca tttcattcac gaccgctttt cgcaattcat ttttaaagta     1020
actgactcag aagggcaacc tgttacagat tatgatttaa tttttacagc cgggccacaa     1080
aacgatgcga accacttacc ggaaggattt gccattgaca ggcaacaaaa ttcaaataat     1140
aacgaaacca ttacgtatta ttttaattac gatgtattga aaggggctcc cgcaaatgtt     1200
taccgggacg cattaccagg tatttctatg ctggggctaa ccataaaccc aaggccggac     1260
gaaggttttg taagatatat cccatgcagc attaaagcca attccgagtt gatggaaaaa     1320
gcctttaaac caaattctac taccttggtc gatattgtta ttcaacgtgt agttagcaaa     1380
gaagtttttc ggttggaaaa gttaactggt agctcaatgc caacagacaa agatgggaat     1440
tttaaaaata ctgaacctgg taacgaaata atatga                               1476
```

<210> 48
<211> 491
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 48

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Met Ser Thr Lys Val Val Phe Val His Gly Trp Ser Val Thr Asn Leu
1               5                   10                  15
Asn Thr Tyr Gly Glu Leu Pro Leu Arg Leu Lys Ala Glu Ala Ile Ser
            20                  25                  30
Arg Asn Leu Asn Ile Glu Val Asn Glu Ile Phe Leu Gly Arg Tyr Ile
        35                  40                  45
Ser Phe Asn Asp Asn Ile Thr Leu Asp Asp Val Ser Arg Ala Phe Asn
    50                  55                  60
Thr Ala Ile Ser Glu Gln Leu Asp Asn Thr Asp Arg Phe Ile Cys Ile
65                  70                  75                  80
Thr His Ser Thr Gly Gly Pro Val Ile Arg Glu Trp Leu Asn Lys Tyr
                85                  90                  95
Tyr Tyr Asn Glu Arg Pro Pro Leu Ser His Leu Ile Met Leu Ala Pro
            100                 105                 110
Ala Asn Phe Gly Ser Ala Leu Ala Arg Leu Gly Lys Ser Lys Leu Ser
        115                 120                 125
Arg Ile Lys Ser Trp Phe Glu Gly Val Glu Pro Gly Gln Lys Ile Leu
    130                 135                 140
Asp Trp Leu Glu Cys Gly Ser Asn Gln Ser Trp Leu Leu Asn Lys Asp
145                 150                 155                 160
Trp Ile Asp Asn Gly Asn Phe Gln Ile Gly Ala Asp Lys Tyr Phe Pro
                165                 170                 175
Phe Val Ile Ile Gly Gln Ser Ile Asp Arg Lys Leu Tyr Asp His Leu
            180                 185                 190
Asn Ser Tyr Thr Gly Glu Leu Gly Ser Asp Gly Val Val Arg Thr Ser
        195                 200                 205
Gly Ala Asn Leu Asn Ser Arg Tyr Ile Lys Leu Val Gln Asp Arg Asn
    210                 215                 220
Thr Ile Ala Asn Gly Asn Ile Ser Ser Thr Leu Arg Ile Ala Glu Tyr
225                 230                 235                 240
Arg Glu Ala Cys Ala Thr Pro Ile Arg Val Val Arg Gly Lys Ser His
                245                 250                 255
Ser Gly Asp Glu Met Gly Ile Met Lys Ser Val Lys Lys Glu Ile Thr
            260                 265                 270
```

Asp Ala Gly Ser Lys Glu Thr Ile Asn Ala Ile Phe Glu Cys Ile Glu
275 280 285
Val Thr Asn Asn Glu Gln Tyr Gln Ser Leu Ile Thr Lys Phe Asp Asn
290 295 300
Glu Thr Ala Gln Val Gln Lys Asp Glu Leu Ile Glu Thr Glu Thr Glu
305 310 315 320
Leu Phe Leu Met His Arg His Phe Ile His Asp Arg Phe Ser Gln Phe
325 330 335
Ile Phe Lys Val Thr Asp Ser Glu Gly Gln Pro Val Thr Asp Tyr Asp
340 345 350
Leu Ile Phe Thr Ala Gly Pro Gln Asn Asp Ala Asn His Leu Pro Glu
355 360 365
Gly Phe Ala Ile Asp Arg Gln Gln Asn Ser Asn Asn Asn Glu Thr Ile
370 375 380
Thr Tyr Tyr Phe Asn Tyr Asp Val Leu Lys Gly Ala Pro Ala Asn Val
385 390 395 400
Tyr Arg Asp Ala Leu Pro Gly Ile Ser Met Leu Gly Leu Thr Ile Asn
405 410 415
Pro Arg Pro Asp Glu Gly Phe Val Arg Tyr Ile Pro Cys Ser Ile Lys
420 425 430
Ala Asn Ser Glu Leu Met Glu Lys Ala Phe Lys Pro Asn Ser Thr Thr
435 440 445
Leu Val Asp Ile Val Ile Gln Arg Val Val Ser Lys Glu Val Phe Arg
450 455 460
Leu Glu Lys Leu Thr Gly Ser Ser Met Pro Thr Asp Lys Asp Gly Asn
465 470 475 480
Phe Lys Asn Thr Glu Pro Gly Asn Glu Ile Ile
485 490

<210> 49
<211> 1257
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 49
atgaattttt ggtcctttct tcttagtata accttaccta tgggggtagg cgttgctcat 60
gcacagcccg atacggattt tcaatcggct gagccttatg tctcttctgc gccaatgggg 120
cgacaaactt atacttacgt gcgttgttgg tatcgcacca gccacagtac ggatgatcca 180
gcgacagatt ggcagtgggc gagaaactcc gatggtagct attttacttt gcaaggatac 240
tggtggagct cggtaagact aaaaaatatg ttttacactc aaacctcgca aaatgttatt 300
cgtcagcgct gcgaacacac tttaagcatt aatcatgata atgcggatat tactttttat 360
gcggcggata atcgtttctc attaaaccat acgatttggt cgaatgatcc tgtcatgcag 420
gctaatcaaa tcaacaagat tgtcgcgttt ggtgacagct tgtccgatac cggtaatatt 480
tttaatgccg cgcagtggcg ttttcctaat cccaatagtt ggtttttggg gcattttttct 540
aacggtttgg tatggactga gtacttagct aaacagaaaa acttaccgat atataactgg 600
gcggttggtg gcgctgctgg ggcgaatcaa tatgtggcgt taaccggtgt tacaggccaa 660
gtgaactctt atttacagta catgggtaaa gcgcaaaact atcgtccaca gaataccttg 720
tacactttgg tcttcggttt gaatgatttt atgaattata accgtgaggt tgctgaggtg 780
gcggctgatt ttgaaacggc attacagcgt ttaacgcaag ctggcgcgca aaatatttta 840
atgatgacgc taccggatgt gactaaagca ccacagttta cctactcaac tcaagcggaa 900
atcgacttga ttcaaggtaa aatcaatgcg ttgaacatca agttaaaaca gttgactgcg 960
caatatattt tacaaggcta tgccattcat ctatttgata cttatgagtt atttgattca 1020
atggtcgctg aaccggaaaa gcatggcttt gctaatgcca gtgaaccttg tttgaatctc 1080
acccgttctt cagcggcgga ttatttgtac cgtcatccca ttaccaatac ttgtgctcgt 1140

tatggtgcag acaaatttgt attttgggat gtcacccatc caaccacggc aactcatcgc 1200
tatatttcac aaacgctgtt agcgccgggt aatggattac aatattttaa tttttaa 1257

<210> 50
<211> 418
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(23)

<400> 50
Met Asn Phe Trp Ser Phe Leu Leu Ser Ile Thr Leu Pro Met Gly Val
1 5 10 15
Gly Val Ala His Ala Gln Pro Asp Thr Asp Phe Gln Ser Ala Glu Pro
20 25 30
Tyr Val Ser Ser Ala Pro Met Gly Arg Gln Thr Tyr Thr Tyr Val Arg
35 40 45
Cys Trp Tyr Arg Thr Ser His Ser Thr Asp Asp Pro Ala Thr Asp Trp
50 55 60
Gln Trp Ala Arg Asn Ser Asp Gly Ser Tyr Phe Thr Leu Gln Gly Tyr
65 70 75 80
Trp Trp Ser Ser Val Arg Leu Lys Asn Met Phe Tyr Thr Gln Thr Ser
85 90 95
Gln Asn Val Ile Arg Gln Arg Cys Glu His Thr Leu Ser Ile Asn His
100 105 110
Asp Asn Ala Asp Ile Thr Phe Tyr Ala Ala Asp Asn Arg Phe Ser Leu
115 120 125
Asn His Thr Ile Trp Ser Asn Asp Pro Val Met Gln Ala Asn Gln Ile
130 135 140
Asn Lys Ile Val Ala Phe Gly Asp Ser Leu Ser Asp Thr Gly Asn Ile
145 150 155 160
Phe Asn Ala Ala Gln Trp Arg Phe Pro Asn Pro Asn Ser Trp Phe Leu
165 170 175
Gly His Phe Ser Asn Gly Leu Val Trp Thr Glu Tyr Leu Ala Lys Gln
180 185 190
Lys Asn Leu Pro Ile Tyr Asn Trp Ala Val Gly Gly Ala Ala Gly Ala
195 200 205
Asn Gln Tyr Val Ala Leu Thr Gly Val Thr Gly Gln Val Asn Ser Tyr
210 215 220
Leu Gln Tyr Met Gly Lys Ala Gln Asn Tyr Arg Pro Gln Asn Thr Leu
225 230 235 240
Tyr Thr Leu Val Phe Gly Leu Asn Asp Phe Met Asn Tyr Asn Arg Glu
245 250 255
Val Ala Glu Val Ala Ala Asp Phe Glu Thr Ala Leu Gln Arg Leu Thr
260 265 270
Gln Ala Gly Ala Gln Asn Ile Leu Met Met Thr Leu Pro Asp Val Thr
275 280 285
Lys Ala Pro Gln Phe Thr Tyr Ser Thr Gln Ala Glu Ile Asp Leu Ile
290 295 300
Gln Gly Lys Ile Asn Ala Leu Asn Ile Lys Leu Lys Gln Leu Thr Ala
305 310 315 320
Gln Tyr Ile Leu Gln Gly Tyr Ala Ile His Leu Phe Asp Thr Tyr Glu
325 330 335

Leu Phe Asp Ser Met Val Ala Glu Pro Glu Lys His Gly Phe Ala Asn
340 345 350
Ala Ser Glu Pro Cys Leu Asn Leu Thr Arg Ser Ser Ala Ala Asp Tyr
355 360 365
Leu Tyr Arg His Pro Ile Thr Asn Thr Cys Ala Arg Tyr Gly Ala Asp
370 375 380
Lys Phe Val Phe Trp Asp Val Thr His Pro Thr Thr Ala Thr His Arg
385 390 395 400
Tyr Ile Ser Gln Thr Leu Leu Ala Pro Gly Asn Gly Leu Gln Tyr Phe
405 410 415
Asn Phe

<210> 51
<211> 1482
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 51
atgacaatcc gctcaacgga ctatgcgctg ctcgcgcagg agagctacca cgacagccag 60
gtcgatgccg acgtcaaact cgatggcatc gcctacaagg tcttcgccac caccgatgac 120
ccgctcacgg ggttccaggc caccgcgtac cagcgccagg acaccggcga agtcgtcatc 180
gcctatcgtg gtacggaatt cgaccgcgag cccgttcgcg acggcggcgt cgatgccggc 240
atggtgctgc tgggggtgaa tgcccagtcg cctgcctccg agctatttac ccgcgaagtg 300
atcgagaagg cgacgcacga agccgaactc aatgaccgcg agccccggat caccgtgact 360
ggccactccc tcggcggcac cctcgccgaa atcaacgcgg ccaagtacgg cctgcacggc 420
gaaaccttca acgcatacgg tgcggccagc ctcaagggca tcccggaagg cggcaatacc 480
gtgatcgacc acgtgcgcgc tggcgacctc gtcagcgccg ccagcccgca ttacgggcag 540
gtgcgcgtct acgcggccca gcaggatatc gacaccttgc agcatgccgg ctaccgcgac 600
gacagcggca tccttagcct gcgcaacccg atcaaggcca cggatttcga cgcgcacgcc 660
atcgacaact tcgtgccgaa cagcaaactg cttggccagt cgatcatcgc gccggaaaac 720
gaagcccgtt acgaagccca caagggcatg gtcgaccgct accgcgatga cgtggctgac 780
atccgcatgc tcgtctccgc tcccctgaac atcccgcgca ccatcggcga tatcaaggat 840
gccgtggaac gcgaggcatt tgagctggct ggcaagggca tcctcgccgt tgaacacggc 900
atcgaagagg tcgtgcacga ggcaaaggaa ggcttcgagc acctcaagga aggctttgag 960
cacctgaagg aagaagtcag cgagggcttc catgccttcg aggaaaaggc ctccagcgcg 1020
tggcatacgc tgacccatcc caaggaatgg ttcgagcacg acaagccgca ggtcgccctg 1080
aaccacccac agcacccgga caacgaactg ttcaagaagg tgctcgaagg cgtgcaccag 1140
gttgatgcga agcagggtcg ttcacccgac cagctcagtg agaacctggc cgcatcgctt 1200
accgttgccg cacgcaagga aggcctggac aaggtcaacc acgtgctgct cgacgacccc 1260
ggcattcgca cctacgccgt gcagggtgag ctcaactcgc cgttgaagca ggtctccagt 1320
gtcgataacg cccaggcggt cgccacaccg gtggcccaga gcagcgcgca atggcagcag 1380
gctgccgagg cgcggcaggc acagcacaat gaggcgcttg cgcagcagca ggcgcaacag 1440
cagcagaaca accggcccaa ccatggggtt gccggcccgt ga 1482

<210> 52
<211> 493
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 52

Met Thr Ile Arg Ser Thr Asp Tyr Ala Leu Leu Ala Gln Glu Ser Tyr
1 5 10 15
His Asp Ser Gln Val Asp Ala Asp Val Lys Leu Asp Gly Ile Ala Tyr
20 25 30
Lys Val Phe Ala Thr Thr Asp Asp Pro Leu Thr Gly Phe Gln Ala Thr
35 40 45
Ala Tyr Gln Arg Gln Asp Thr Gly Glu Val Val Ile Ala Tyr Arg Gly
50 55 60
Thr Glu Phe Asp Arg Glu Pro Val Arg Asp Gly Gly Val Asp Ala Gly
65 70 75 80
Met Val Leu Leu Gly Val Asn Ala Gln Ser Pro Ala Ser Glu Leu Phe
85 90 95
Thr Arg Glu Val Ile Glu Lys Ala Thr His Glu Ala Glu Leu Asn Asp
100 105 110
Arg Glu Pro Arg Ile Thr Val Thr Gly His Ser Leu Gly Gly Thr Leu
115 120 125
Ala Glu Ile Asn Ala Ala Lys Tyr Gly Leu His Gly Glu Thr Phe Asn
130 135 140
Ala Tyr Gly Ala Ala Ser Leu Lys Gly Ile Pro Glu Gly Gly Asn Thr
145 150 155 160
Val Ile Asp His Val Arg Ala Gly Asp Leu Val Ser Ala Ala Ser Pro
165 170 175
His Tyr Gly Gln Val Arg Val Tyr Ala Ala Gln Gln Asp Ile Asp Thr
180 185 190
Leu Gln His Ala Gly Tyr Arg Asp Asp Ser Gly Ile Leu Ser Leu Arg
195 200 205
Asn Pro Ile Lys Ala Thr Asp Phe Asp Ala His Ala Ile Asp Asn Phe
210 215 220
Val Pro Asn Ser Lys Leu Leu Gly Gln Ser Ile Ile Ala Pro Glu Asn
225 230 235 240
Glu Ala Arg Tyr Glu Ala His Lys Gly Met Val Asp Arg Tyr Arg Asp
245 250 255
Asp Val Ala Asp Ile Arg Met Leu Val Ser Ala Pro Leu Asn Ile Pro
260 265 270
Arg Thr Ile Gly Asp Ile Lys Asp Ala Val Glu Arg Glu Ala Phe Glu
275 280 285
Leu Ala Gly Lys Gly Ile Leu Ala Val Glu His Gly Ile Glu Glu Val
290 295 300
Val His Glu Ala Lys Glu Gly Phe Glu His Leu Lys Glu Gly Phe Glu
305 310 315 320
His Leu Lys Glu Glu Val Ser Glu Gly Phe His Ala Phe Glu Glu Lys
325 330 335
Ala Ser Ser Ala Trp His Thr Leu Thr His Pro Lys Glu Trp Phe Glu
340 345 350
His Asp Lys Pro Gln Val Ala Leu Asn His Pro Gln His Pro Asp Asn
355 360 365
Glu Leu Phe Lys Lys Val Leu Glu Gly Val His Gln Val Asp Ala Lys
370 375 380
Gln Gly Arg Ser Pro Asp Gln Leu Ser Glu Asn Leu Ala Ala Ser Leu
385 390 395 400
Thr Val Ala Ala Arg Lys Glu Gly Leu Asp Lys Val Asn His Val Leu
405 410 415
Leu Asp Asp Pro Gly Ile Arg Thr Tyr Ala Val Gln Gly Glu Leu Asn
420 425 430
Ser Pro Leu Lys Gln Val Ser Ser Val Asp Asn Ala Gln Ala Val Ala
435 440 445
Thr Pro Val Ala Gln Ser Ser Ala Gln Trp Gln Gln Ala Ala Glu Ala

450 455 460
Arg Gln Ala Gln His Asn Glu Ala Leu Ala Gln Gln Gln Ala Gln Gln
465 470 475 480
Gln Gln Asn Asn Arg Pro Asn His Gly Val Ala Gly Pro
485 490

<210> 53
<211> 1491
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 53
atgcgtcagg ttacattagt atttgttcat ggctacagcg ttacaaacat cgacacttat 60
ggtgaaatgc cactcaggct ccgcaacgaa ggagccacac gtgatataga aataaaaatt 120
gagaacattt tcctggggcg ctacatcagc tttaatgatg atgtgagatt aaatgatgtt 180
tccagagcat tggaaacagc cgtacaacaa cagattgcac cgggaaataa aaacaattcc 240
cgttacgtat tcatcaccca ctctaccggc ggaccggtag tgagaaactg gtgggatctg 300
tactataaaa acagcacgaa acaatgccct atgagccacc tcattatgct ggctcctgcc 360
aattttggct cggcactggc acaactggga aaaagcaaac taagccgcat taaatcctgg 420
ttcgatggtg tggaacccgg acagaatgta ttgaattggc tggaactggg aagcgcggaa 480
gcatggaagc taaacaccga ctggattaag agtgatggaa gtcagatctc ggcacagggt 540
atttttcctt ttgtgatcat aggtcaggac attgaccgca aattatacga tcatttaaac 600
tcctacaccg gtgagctggg ttccgacggc gtggtgcgtt cggccgcagc caatttaaat 660
gctacttatg taaaactcac acaacctaaa cccaccttgg taaatggaaa actggtaaca 720
ggtaatctgg aaataggaga agtaaaacaa gcgccttata cacccatgcg catcgtctca 780
aaaaaatcgc attccaacaa ggatatggga attatgagaa gtgtactgaa atcaacaaat 840
gatgccaaca gcgccgaaac ggtaaacgcc atttttgact gcattaatgt gaaaacctta 900
accgattacc agagcattgc cacacagttt gattcgcaaa caaaagacgt gcaggaaaat 960
tcaattattg aaagggaaaa aacgcccttt ggaactaaaa actatattca cgaccgtttc 1020
tcccaggtca ttttcagagt aacagacagt gaaggttacc cggttaccag ttttgatctg 1080
atcctcaccg gcggcgaaaa aaatgatccc aacgccttgc ctcagggctt ttttgtggac 1140
agacaatgca acagtgtcaa taaatcgacc attacttatt ttttaaatta cgatattatg 1200
aacggcacac cagctatagc aggtataaga ccggcatcca aaggcatgga aaaactgggt 1260
ctgatcatta acccaaggcc tgaagaaggc tttgtgcgtt acattccctg caaaataaac 1320
acatcgcccg atttgtttga cgccgctctg aaacccaacg ccacaacgct tattgatatt 1380
gtattgcaac gcgtggtaag taccgaagta ttccgctttg aaggaacaga cggggtaacg 1440
ccgcctaaaa aagatttctc gaaagtgaaa cccggaacgg atattatttg a 1491

<210> 54
<211> 496
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 54
Met Arg Gln Val Thr Leu Val Phe Val His Gly Tyr Ser Val Thr Asn
1 5 10 15
Ile Asp Thr Tyr Gly Glu Met Pro Leu Arg Leu Arg Asn Glu Gly Ala
20 25 30
Thr Arg Asp Ile Glu Ile Lys Ile Glu Asn Ile Phe Leu Gly Arg Tyr
35 40 45
Ile Ser Phe Asn Asp Asp Val Arg Leu Asn Asp Val Ser Arg Ala Leu

```
    50                  55                  60
Glu Thr Ala Val Gln Gln Gln Ile Ala Pro Gly Asn Lys Asn Asn Ser
65                  70                  75                  80
Arg Tyr Val Phe Ile Thr His Ser Thr Gly Gly Pro Val Val Arg Asn
                85                  90                  95
Trp Trp Asp Leu Tyr Tyr Lys Asn Ser Thr Lys Gln Cys Pro Met Ser
            100                 105                 110
His Leu Ile Met Leu Ala Pro Ala Asn Phe Gly Ser Ala Leu Ala Gln
        115                 120                 125
Leu Gly Lys Ser Lys Leu Ser Arg Ile Lys Ser Trp Phe Asp Gly Val
    130                 135                 140
Glu Pro Gly Gln Asn Val Leu Asn Trp Leu Glu Leu Gly Ser Ala Glu
145                 150                 155                 160
Ala Trp Lys Leu Asn Thr Asp Trp Ile Lys Ser Asp Gly Ser Gln Ile
                165                 170                 175
Ser Ala Gln Gly Ile Phe Pro Phe Val Ile Ile Gly Gln Asp Ile Asp
            180                 185                 190
Arg Lys Leu Tyr Asp His Leu Asn Ser Tyr Thr Gly Glu Leu Gly Ser
        195                 200                 205
Asp Gly Val Val Arg Ser Ala Ala Ala Asn Leu Asn Ala Thr Tyr Val
    210                 215                 220
Lys Leu Thr Gln Pro Lys Pro Thr Leu Val Asn Gly Lys Leu Val Thr
225                 230                 235                 240
Gly Asn Leu Glu Ile Gly Glu Val Lys Gln Ala Pro Tyr Thr Pro Met
                245                 250                 255
Arg Ile Val Ser Lys Lys Ser His Ser Asn Lys Asp Met Gly Ile Met
            260                 265                 270
Arg Ser Val Leu Lys Ser Thr Asn Asp Ala Asn Ser Ala Glu Thr Val
        275                 280                 285
Asn Ala Ile Phe Asp Cys Ile Asn Val Lys Thr Leu Thr Asp Tyr Gln
    290                 295                 300
Ser Ile Ala Thr Gln Phe Asp Ser Gln Thr Lys Asp Val Gln Glu Asn
305                 310                 315                 320
Ser Ile Ile Glu Arg Glu Lys Thr Pro Phe Gly Thr Lys Asn Tyr Ile
                325                 330                 335
His Asp Arg Phe Ser Gln Val Ile Phe Arg Val Thr Asp Ser Glu Gly
            340                 345                 350
Tyr Pro Val Thr Ser Phe Asp Leu Ile Leu Thr Gly Gly Glu Lys Asn
        355                 360                 365
Asp Pro Asn Ala Leu Pro Gln Gly Phe Phe Val Asp Arg Gln Cys Asn
    370                 375                 380
Ser Val Asn Lys Ser Thr Ile Thr Tyr Phe Leu Asn Tyr Asp Ile Met
385                 390                 395                 400
Asn Gly Thr Pro Ala Ile Ala Gly Ile Arg Pro Ala Ser Lys Gly Met
                405                 410                 415
Glu Lys Leu Gly Leu Ile Ile Asn Pro Arg Pro Glu Glu Gly Phe Val
            420                 425                 430
Arg Tyr Ile Pro Cys Lys Ile Asn Thr Ser Pro Asp Leu Phe Asp Ala
        435                 440                 445
Ala Leu Lys Pro Asn Ala Thr Thr Leu Ile Asp Ile Val Leu Gln Arg
    450                 455                 460
Val Val Ser Thr Glu Val Phe Arg Phe Glu Gly Thr Asp Gly Val Thr
465                 470                 475                 480
Pro Pro Lys Lys Asp Phe Ser Lys Val Lys Pro Gly Thr Asp Ile Ile
                485                 490                 495
```

<210> 55

<211> 1041
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 55
```
atggcttcac aattcagaaa tctggttttt gaaggaggcg gtgtgaaggg catcgcctat     60
atcggcgcca tgcaggtgct ggagcagcgg ggactgctca aggatattgt ccgggtggga    120
ggtaccagtg caggcgccat caacgcgctg atcttttcgc tgggctttac catcaaagag    180
cagcaggata ttctcaactc caccaacttc agggagttta tggacagctc gttcgggttc    240
atccgaaact tccggaggtt atggagcgaa ttcggttgga accgcggcga tgtattttcg    300
gactgggccg gggagctggt gaaagagaag ctcggcaaaa agaacgccac gttcggcgat    360
ctgaaaaagg cgaaacgtcc cgatctgtac gtgatcggca ccaatctctc tacggggttt    420
tccgagacct tttcgcacga acgccacgcc gacatgcctc tggtagatgc ggtgcggata    480
agcatgtcga tcccgctctt ttttgctgca cggaggctgg gaaaacgtaa ggatgtgtat    540
gtggatggcg ggtgatgct caactatccc gtgaagctgt tcgacaggga gaagtatatc     600
gatttggaga aagagaatga ggcggcccgc tatgtggagt actacaatca agagaatgcc    660
cggtttctgc tcgagcggcc cggccgaagc ccttatgtgt ataaccggca gactctcggt    720
ctgcggctcg acacgcagga agagatcggc ctgttccgtt acgatgagcc gctgaagggc    780
aagcagatca accgtttccc cgaatacgcc agagccctga tcggctcgct gatgcaggta    840
caggagaaca tccacctgaa aagtgacgac tggcagcgaa cgctctacat caacacgctg    900
gatgtgggca ccaccgattt cgacattacc gacgagaaga aaaaagtgct ggtgaatgag    960
gggatcaagg gagcggagac ctatttccgc tggtttgagg atcccgaaga aaaaccggtg   1020
aataaggtga atcttgtctg a                                             1041
```

<210> 56
<211> 346
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 56
```
Met Ala Ser Gln Phe Arg Asn Leu Val Phe Glu Gly Gly Gly Val Lys
1               5                   10                  15
Gly Ile Ala Tyr Ile Gly Ala Met Gln Val Leu Glu Gln Arg Gly Leu
            20                  25                  30
Leu Lys Asp Ile Val Arg Val Gly Gly Thr Ser Ala Gly Ala Ile Asn
        35                  40                  45
Ala Leu Ile Phe Ser Leu Gly Phe Thr Ile Lys Glu Gln Gln Asp Ile
    50                  55                  60
Leu Asn Ser Thr Asn Phe Arg Glu Phe Met Asp Ser Ser Phe Gly Phe
65                  70                  75                  80
Ile Arg Asn Phe Arg Arg Leu Trp Ser Glu Phe Gly Trp Asn Arg Gly
                85                  90                  95
Asp Val Phe Ser Asp Trp Ala Gly Glu Leu Val Lys Glu Lys Leu Gly
            100                 105                 110
Lys Lys Asn Ala Thr Phe Gly Asp Leu Lys Lys Ala Lys Arg Pro Asp
        115                 120                 125
Leu Tyr Val Ile Gly Thr Asn Leu Ser Thr Gly Phe Ser Glu Thr Phe
    130                 135                 140
Ser His Glu Arg His Ala Asp Met Pro Leu Val Asp Ala Val Arg Ile
145                 150                 155                 160
Ser Met Ser Ile Pro Leu Phe Phe Ala Ala Arg Arg Leu Gly Lys Arg
```

165 170 175
Lys Asp Val Tyr Val Asp Gly Gly Val Met Leu Asn Tyr Pro Val Lys
180 185 190
Leu Phe Asp Arg Glu Lys Tyr Ile Asp Leu Glu Lys Glu Asn Glu Ala
195 200 205
Ala Arg Tyr Val Glu Tyr Tyr Asn Gln Glu Asn Ala Arg Phe Leu Leu
210 215 220
Glu Arg Pro Gly Arg Ser Pro Tyr Val Tyr Asn Arg Gln Thr Leu Gly
225 230 235 240
Leu Arg Leu Asp Thr Gln Glu Glu Ile Gly Leu Phe Arg Tyr Asp Glu
245 250 255
Pro Leu Lys Gly Lys Gln Ile Asn Arg Phe Pro Glu Tyr Ala Arg Ala
260 265 270
Leu Ile Gly Ser Leu Met Gln Val Gln Glu Asn Ile His Leu Lys Ser
275 280 285
Asp Asp Trp Gln Arg Thr Leu Tyr Ile Asn Thr Leu Asp Val Gly Thr
290 295 300
Thr Asp Phe Asp Ile Thr Asp Glu Lys Lys Lys Val Leu Val Asn Glu
305 310 315 320
Gly Ile Lys Gly Ala Glu Thr Tyr Phe Arg Trp Phe Glu Asp Pro Glu
325 330 335
Glu Lys Pro Val Asn Lys Val Asn Leu Val
340 345

<210> 57
<211> 1413
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 57
atgcaattag tgttcgtaca cgggtggagt gttacccata ccaataccta tggtgaatta 60
cccgaaagtt tggcggcagg cgccgcgaca cacggcctgc agatcgatat caggcacgtt 120
tttctcggca agtacatcag ctttcacgat gaggtgactc tggatgatat agcacgtgcc 180
ttcgacaagg cgctgagaga catgtcgggt gatggtgaca cggtctcgcc tttctcctgt 240
atcacgcatt cgaccggcgg ccctgtcgtt cggcactgga ttaacaaatt ctacggcgcg 300
cgagggctat cgaaactgcc gctggagcat ttggttatgc tggcgcctgc caaccacggc 360
tccagcctgg cggtactcgg caagcaacgt cttggtcgca tcaagtcctg gttcgatggc 420
gtggagcccg gacaaaaagt gctcgactgg ctatcgctgg gcagcaatgg gcaatgggcg 480
ctcaacaggg atttttttgag ctaccgcccg gccaaacatg gcttcttccc ttttgttctg 540
acgggccagg gtatagacac aaaattctac gattttttga acagctacct tgtggagccc 600
ggcagtgacg gtgtggttcg cgtggcgggt gccaatatgc attttcgcta cctctccctg 660
gtacaatctg agaccgtatt acacaccccg ggcaaggtgc tacagctgga atataacgag 720
cggcgccccg tgaagtcccc acaagcggta ccgatgggcg tcttctccca atttagccac 780
tctggcgaca agatggggat tatggcagtc aagcgcaaga aagacgcgca tcaaatgatc 840
gtaacggaag tgctgaagtg tctctgcgta tcggacagcg atgaatatca gcaaagaggc 900
cttgaacttg cagaactgac cgccagcgaa cagcgcaagc ccatcgaaga ccaggacaag 960
attatcagcc gctatagcat gctggtattt agagtgcgcg accaggcggg caatacgatc 1020
ggagtgcacg atttcgatat cctcttactg gccggagata cctatagccc cgacaaactg 1080
ccagaggggt tcttcatgga taaacaggcc aatagagatg ccggctcact gatctactat 1140
gtggatgccg acaaaatgtc cgagatgaaa gatggctgct acggactgcg ggtggtcgtg 1200
cggccggaga aagggttttc ctattacaca acaggtgagt tcaggtcaga gggtatcccc 1260
gtggaccgtg tatttgcagc aaacgaaacc acctatattg atatcaccat gaaccgaagt 1320
gtcgatcaaa atgtattccg gttttcgcct gcaacagagc cacctgaaag cttcaaaaga 1380
accacgccct caggtaccga tatcccttca tag 1413

<210> 58
<211> 470
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 58
Met Gln Leu Val Phe Val His Gly Trp Ser Val Thr His Thr Asn Thr
1 5 10 15
Tyr Gly Glu Leu Pro Glu Ser Leu Ala Ala Gly Ala Ala Thr His Gly
20 25 30
Leu Gln Ile Asp Ile Arg His Val Phe Leu Gly Lys Tyr Ile Ser Phe
35 40 45
His Asp Glu Val Thr Leu Asp Asp Ile Ala Arg Ala Phe Asp Lys Ala
50 55 60
Leu Arg Asp Met Ser Gly Asp Gly Asp Thr Val Ser Pro Phe Ser Cys
65 70 75 80
Ile Thr His Ser Thr Gly Gly Pro Val Val Arg His Trp Ile Asn Lys
85 90 95
Phe Tyr Gly Ala Arg Gly Leu Ser Lys Leu Pro Leu Glu His Leu Val
100 105 110
Met Leu Ala Pro Ala Asn His Gly Ser Ser Leu Ala Val Leu Gly Lys
115 120 125
Gln Arg Leu Gly Arg Ile Lys Ser Trp Phe Asp Gly Val Glu Pro Gly
130 135 140
Gln Lys Val Leu Asp Trp Leu Ser Leu Gly Ser Asn Gly Gln Trp Ala
145 150 155 160
Leu Asn Arg Asp Phe Leu Ser Tyr Arg Pro Ala Lys His Gly Phe Phe
165 170 175
Pro Phe Val Leu Thr Gly Gln Gly Ile Asp Thr Lys Phe Tyr Asp Phe
180 185 190
Leu Asn Ser Tyr Leu Val Glu Pro Gly Ser Asp Gly Val Val Arg Val
195 200 205
Ala Gly Ala Asn Met His Phe Arg Tyr Leu Ser Leu Val Gln Ser Glu
210 215 220
Thr Val Leu His Thr Pro Gly Lys Val Leu Gln Leu Glu Tyr Asn Glu
225 230 235 240
Arg Arg Pro Val Lys Ser Pro Gln Ala Val Pro Met Gly Val Phe Ser
245 250 255
Gln Phe Ser His Ser Gly Asp Lys Met Gly Ile Met Ala Val Lys Arg
260 265 270
Lys Lys Asp Ala His Gln Met Ile Val Thr Glu Val Leu Lys Cys Leu
275 280 285
Cys Val Ser Asp Ser Asp Glu Tyr Gln Gln Arg Gly Leu Glu Leu Ala
290 295 300
Glu Leu Thr Ala Ser Glu Gln Arg Lys Pro Ile Glu Asp Gln Asp Lys
305 310 315 320
Ile Ile Ser Arg Tyr Ser Met Leu Val Phe Arg Val Arg Asp Gln Ala
325 330 335
Gly Asn Thr Ile Gly Val His Asp Phe Asp Ile Leu Leu Leu Ala Gly
340 345 350
Asp Thr Tyr Ser Pro Asp Lys Leu Pro Glu Gly Phe Phe Met Asp Lys
355 360 365
Gln Ala Asn Arg Asp Ala Gly Ser Leu Ile Tyr Tyr Val Asp Ala Asp

```
    370                 375                 380
Lys Met Ser Glu Met Lys Asp Gly Cys Tyr Gly Leu Arg Val Val Val
385                 390                 395                 400
Arg Pro Glu Lys Gly Phe Ser Tyr Tyr Thr Thr Gly Glu Phe Arg Ser
                405                 410                 415
Glu Gly Ile Pro Val Asp Arg Val Phe Ala Ala Asn Glu Thr Thr Tyr
            420                 425                 430
Ile Asp Ile Thr Met Asn Arg Ser Val Asp Gln Asn Val Phe Arg Phe
        435                 440                 445
Ser Pro Ala Thr Glu Pro Pro Glu Ser Phe Lys Arg Thr Thr Pro Ser
    450                 455                 460
Gly Thr Asp Ile Pro Ser
465                 470
```

<210> 59
<211> 1038
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 59

```
atgacaacac aatttagaaa cttgatcttt gaaggcggcg gtgtaaaagg cgttgcttac      60
attggcgcca tgcagattct tgaaaatcgt ggcgtgttgc aagatattcg ccgagtcgga     120
gggtgcagtg cgggtgcgat taacgcgctg atttttgcgc tgggttacac ggtccgtgag     180
caaaaagaga tcttacaagc caccgatttt aaccagttta tggataactc ttggggggtt     240
attcgtgata ttcgcaggct tgctcgagac tttggctgga ataagggtga tttctttagt     300
agctggatag gtgatttgat tcatcgtcgt ttggggaatc gccgagcgac gttcaaagat     360
ctgcaaaagg ccaagcttcc tgatctttat gtcatcggta ctaatctgtc tacagggttt     420
gcagaggtgt tttctgccga aagacacccc gatatggagc tggcgacagc ggtgcgtatc     480
tccatgtcga taccgctgtt ctttgcggca gtgcgtcatg gtgatcgaca agatgtgtat     540
gtcgatgggg gtgttcaact taactatccg attaaactgt ttgatcggga gcgttatatt     600
gatctggcca aagatcccgg tgccgttcgg cgaacgggtt attacaacaa agaaaacgct     660
cgctttcagc ttgatcggcc gggccatagc ccctatgttt acaatcgcca gaccttgggt     720
ttgcgactgg atagtcgcga ggagataggg ctctttcgtt atgacgaacc cctcaagggc     780
aaacccatta agtccttcac tgactacgct cgacaacttt tcggtgcgct gatgaatgca     840
caggaaaaga ttcatctaca tggcgatgat tggcaacgca cggtctatat cgatacactc     900
gatgtgggta cgacggactt caatctttct gatgcaacca agcaagcact gattgagcaa     960
ggaattaacg gcaccgaaaa ttatttcgac tggtttgata atccgttaga gaagcctgtg    1020
aatagagtgg agtcatag                                                 1038
```

<210> 60
<211> 345
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 60

```
Met Thr Thr Gln Phe Arg Asn Leu Ile Phe Glu Gly Gly Gly Val Lys
 1               5                  10                  15
Gly Val Ala Tyr Ile Gly Ala Met Gln Ile Leu Glu Asn Arg Gly Val
            20                  25                  30
Leu Gln Asp Ile Arg Arg Val Gly Gly Cys Ser Ala Gly Ala Ile Asn
        35                  40                  45
```

Ala Leu Ile Phe Ala Leu Gly Tyr Thr Val Arg Glu Gln Lys Glu Ile
50 55 60
Leu Gln Ala Thr Asp Phe Asn Gln Phe Met Asp Asn Ser Trp Gly Val
65 70 75 80
Ile Arg Asp Ile Arg Arg Leu Ala Arg Asp Phe Gly Trp Asn Lys Gly
85 90 95
Asp Phe Phe Ser Ser Trp Ile Gly Asp Leu Ile His Arg Arg Leu Gly
100 105 110
Asn Arg Arg Ala Thr Phe Lys Asp Leu Gln Lys Ala Lys Leu Pro Asp
115 120 125
Leu Tyr Val Ile Gly Thr Asn Leu Ser Thr Gly Phe Ala Glu Val Phe
130 135 140
Ser Ala Glu Arg His Pro Asp Met Glu Leu Ala Thr Ala Val Arg Ile
145 150 155 160
Ser Met Ser Ile Pro Leu Phe Phe Ala Ala Val Arg His Gly Asp Arg
165 170 175
Gln Asp Val Tyr Val Asp Gly Gly Val Gln Leu Asn Tyr Pro Ile Lys
180 185 190
Leu Phe Asp Arg Glu Arg Tyr Ile Asp Leu Ala Lys Asp Pro Gly Ala
195 200 205
Val Arg Arg Thr Gly Tyr Tyr Asn Lys Glu Asn Ala Arg Phe Gln Leu
210 215 220
Asp Arg Pro Gly His Ser Pro Tyr Val Tyr Asn Arg Gln Thr Leu Gly
225 230 235 240
Leu Arg Leu Asp Ser Arg Glu Glu Ile Gly Leu Phe Arg Tyr Asp Glu
245 250 255
Pro Leu Lys Gly Lys Pro Ile Lys Ser Phe Thr Asp Tyr Ala Arg Gln
260 265 270
Leu Phe Gly Ala Leu Met Asn Ala Gln Glu Lys Ile His Leu His Gly
275 280 285
Asp Asp Trp Gln Arg Thr Val Tyr Ile Asp Thr Leu Asp Val Gly Thr
290 295 300
Thr Asp Phe Asn Leu Ser Asp Ala Thr Lys Gln Ala Leu Ile Glu Gln
305 310 315 320
Gly Ile Asn Gly Thr Glu Asn Tyr Phe Asp Trp Phe Asp Asn Pro Leu
325 330 335
Glu Lys Pro Val Asn Arg Val Glu Ser
340 345

<210> 61
<211> 1257
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 61
atgacattaa aactctccct gctgatcgcg agcctgagcg ccgtgtctcc agcagtcttg 60
gcaaacgacg tcaatccagc gccactcatg gcgccgtccg aagcggattc cgcgcagacg 120
ctgggcagtc tgacgtacac ctatgttcgc tgctggtatc gtccggctgc gacgcataat 180
gatccttaca ccacctggga gtgggcgaag aacgcggacg gcagtgattt caccattgat 240
ggctattggt ggtcatcggt gagttacaaa aacatgttct ataccgatac tcagcccgat 300
accatcatgc agcgctgtgc agagacgttg gggttaaccc acgataccgc tgacatcacc 360
tatgccgcgg ccgatacccg tttctcctac aaccacacca tctggagcaa cgatgtcgcc 420
aacgcgccga gcaaaatcaa taaggtgatc gcctttggtg acagcctgtc agacacgggc 480
aacattttta acgcctcgca atggcgcttc ccgaacccga actcctggtt tgtcggccac 540

```
ttctcaaacg ggtttgtctg gaccgagtat ctggcgcaag gtttggggct gcccctctac    600
aactgggccg tgggcggcgc ggcggggcgc aatcaatact gggcgctgac tggcgtgaat    660
gaacaggtca gttcgtacct gacctacatg gagatggcgc cgaattaccg tgcggagaac    720
acgctgttta cactcgaatt cggtctgaat gattttatga actacgaccg ttcactggca    780
gacgtcaaag cagattacag ctcggcgctg attcgtctgg tggaagccgg agcgaaaaat    840
atggtgctgt tgacctacc ggatgccacg cgcgcgccgc agttccaata ttcaacgcaa    900
gaacacatcg acgaggtgcg cgccaaagtg attggcatga acgcgttcat tcgtgagcag    960
gcacgctact tccagatgca gggcatcaac atttcgctgt ttgacgccta cacgctgttt   1020
gatcagatga tcgccgaccc agccgcgcac ggctttgata atgccagcgc gccatgtctt   1080
gatattcagc gcagctctgc ggcggactat ctctacacgc atgctctggc agccgagtgt   1140
gcctcatccg gttcagaccg ctttgtgttc tgggatgtga ctcacccaac cacggcaacg   1200
catcgctaca tcgccgacca cattctggct accggtgttg cgcagttccc gcgttaa      1257

<210> 62
<211> 418
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(21)

<400> 62
Met Thr Leu Lys Leu Ser Leu Leu Ile Ala Ser Leu Ser Ala Val Ser
 1               5                  10                  15
Pro Ala Val Leu Ala Asn Asp Val Asn Pro Ala Pro Leu Met Ala Pro
            20                  25                  30
Ser Glu Ala Asp Ser Ala Gln Thr Leu Gly Ser Leu Thr Tyr Thr Tyr
        35                  40                  45
Val Arg Cys Trp Tyr Arg Pro Ala Ala Thr His Asn Asp Pro Tyr Thr
    50                  55                  60
Thr Trp Glu Trp Ala Lys Asn Ala Asp Gly Ser Asp Phe Thr Ile Asp
65                  70                  75                  80
Gly Tyr Trp Trp Ser Ser Val Ser Tyr Lys Asn Met Phe Tyr Thr Asp
                85                  90                  95
Thr Gln Pro Asp Thr Ile Met Gln Arg Cys Ala Glu Thr Leu Gly Leu
            100                 105                 110
Thr His Asp Thr Ala Asp Ile Thr Tyr Ala Ala Ala Asp Thr Arg Phe
        115                 120                 125
Ser Tyr Asn His Thr Ile Trp Ser Asn Asp Val Ala Asn Ala Pro Ser
    130                 135                 140
Lys Ile Asn Lys Val Ile Ala Phe Gly Asp Ser Leu Ser Asp Thr Gly
145                 150                 155                 160
Asn Ile Phe Asn Ala Ser Gln Trp Arg Phe Pro Asn Pro Asn Ser Trp
                165                 170                 175
Phe Val Gly His Phe Ser Asn Gly Phe Val Trp Thr Glu Tyr Leu Ala
            180                 185                 190
Gln Gly Leu Gly Leu Pro Leu Tyr Asn Trp Ala Val Gly Gly Ala Ala
        195                 200                 205
Gly Arg Asn Gln Tyr Trp Ala Leu Thr Gly Val Asn Glu Gln Val Ser
    210                 215                 220
Ser Tyr Leu Thr Tyr Met Glu Met Ala Pro Asn Tyr Arg Ala Glu Asn
225                 230                 235                 240
Thr Leu Phe Thr Leu Glu Phe Gly Leu Asn Asp Phe Met Asn Tyr Asp
                245                 250                 255
```

```
Arg Ser Leu Ala Asp Val Lys Ala Asp Tyr Ser Ser Ala Leu Ile Arg
            260                 265                 270
Leu Val Glu Ala Gly Ala Lys Asn Met Val Leu Leu Thr Leu Pro Asp
        275                 280                 285
Ala Thr Arg Ala Pro Gln Phe Gln Tyr Ser Thr Gln Glu His Ile Asp
    290                 295                 300
Glu Val Arg Ala Lys Val Ile Gly Met Asn Ala Phe Ile Arg Glu Gln
305                 310                 315                 320
Ala Arg Tyr Phe Gln Met Gln Gly Ile Asn Ile Ser Leu Phe Asp Ala
                325                 330                 335
Tyr Thr Leu Phe Asp Gln Met Ile Ala Asp Pro Ala Ala His Gly Phe
            340                 345                 350
Asp Asn Ala Ser Ala Pro Cys Leu Asp Ile Gln Arg Ser Ser Ala Ala
        355                 360                 365
Asp Tyr Leu Tyr Thr His Ala Leu Ala Ala Glu Cys Ala Ser Ser Gly
    370                 375                 380
Ser Asp Arg Phe Val Phe Trp Asp Val Thr His Pro Thr Thr Ala Thr
385                 390                 395                 400
His Arg Tyr Ile Ala Asp His Ile Leu Ala Thr Gly Val Ala Gln Phe
                405                 410                 415
Pro Arg
```

<210> 63
<211> 1242
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 63

```
atgaaaaata cgttaatttt ggctggctgt atattggcag ctccagccgt cgcagatgac      60
ctaacaatca cccctgaaac tataagtgtg cgctacgcgt ctgaggtgca gaacaaacaa     120
acatacactt atgttcgctg ctggtatcgt ccagcgcaga accatgacga cccttccact     180
gagtgggaat gggctcgtga cgacaatggc gattacttca ctatcgatgg gtactggtgg     240
tcgtctgtct ccttcaaaaa catgttctat accaataccc cgcaaacaga aattgaaaac     300
cgctgtaaag aaacactagg ggttaatcat gatagtgccg atcttcttta ctatgcatca     360
gacaatcgtt tctcctacaa ccatagtatt tggacaaacg acaacgcagt aaacaacaaa     420
atcaatcgta ttgtcgcatt cggtgatagc ctgtctgaca ccggtaatct gtacaatgga     480
tcccaatggg tattccccaa ccgtaattct tggtttctcg gtcacttttc aaacggtttg     540
gtgtggactg aatacttagc gcaaaacaaa aacgtaccac tgtacaactg ggcggtcggt     600
ggcgccgccg gcaccaacca atacgtcgca ttgacaggca tttatgacca agtgacgtct     660
tatcttacgt acatgaagat ggcaaagaac tacaacccaa acaacagttt gatgacgctg     720
gaatttggcc taaatgattt catgaattac ggccgagaag tggcggacgt gaaagctgac     780
ttaagtagcg cattgattcg cttgaccgaa tcaggcgcaa gcaacattct actcttcacg     840
ttaccggacg caacaaaggc accgcagttt aaatattcga ctcaggagga aattgagacc     900
gttcgagcta agattcttga gttcaacact tttattgaag aacaagcgtt actctatcaa     960
gctaaaggac tgaatgtggc cctctacgat gctcatagca tctttgatca gctgacatcc    1020
aatcctaaac aacacggttt tgagaactca acagatgcct gtctgaacat caaccgcagt    1080
tcctctgtcg actaccttta cagtcatgag ctaactaacg attgtgcgta tcatagctct    1140
gataaatatg tgttctgggg agtcactcac ccaaccacag caacacataa atacattgcc    1200
gaccaaatca ttcagaccaa gctagaccag ttcaatttct aa                       1242
```

<210> 64
<211> 413
<212> PRT

<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(18)

<400> 64
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Met Lys Asn Thr Leu Ile Leu Ala Gly Cys Ile Leu Ala Ala Pro Ala
 1               5                  10                  15
Val Ala Asp Asp Leu Thr Ile Thr Pro Glu Thr Ile Ser Val Arg Tyr
            20                  25                  30
Ala Ser Glu Val Gln Asn Lys Gln Thr Tyr Thr Tyr Val Arg Cys Trp
        35                  40                  45
Tyr Arg Pro Ala Gln Asn His Asp Asp Pro Ser Thr Glu Trp Glu Trp
    50                  55                  60
Ala Arg Asp Asp Asn Gly Asp Tyr Phe Thr Ile Asp Gly Tyr Trp Trp
65                  70                  75                  80
Ser Ser Val Ser Phe Lys Asn Met Phe Tyr Thr Asn Thr Pro Gln Thr
                85                  90                  95
Glu Ile Glu Asn Arg Cys Lys Glu Thr Leu Gly Val Asn His Asp Ser
            100                 105                 110
Ala Asp Leu Leu Tyr Tyr Ala Ser Asp Asn Arg Phe Ser Tyr Asn His
        115                 120                 125
Ser Ile Trp Thr Asn Asp Asn Ala Val Asn Asn Lys Ile Asn Arg Ile
    130                 135                 140
Val Ala Phe Gly Asp Ser Leu Ser Asp Thr Gly Asn Leu Tyr Asn Gly
145                 150                 155                 160
Ser Gln Trp Val Phe Pro Asn Arg Asn Ser Trp Phe Leu Gly His Phe
                165                 170                 175
Ser Asn Gly Leu Val Trp Thr Glu Tyr Leu Ala Gln Asn Lys Asn Val
            180                 185                 190
Pro Leu Tyr Asn Trp Ala Val Gly Gly Ala Ala Gly Thr Asn Gln Tyr
        195                 200                 205
Val Ala Leu Thr Gly Ile Tyr Asp Gln Val Thr Ser Tyr Leu Thr Tyr
    210                 215                 220
Met Lys Met Ala Lys Asn Tyr Asn Pro Asn Asn Ser Leu Met Thr Leu
225                 230                 235                 240
Glu Phe Gly Leu Asn Asp Phe Met Asn Tyr Gly Arg Glu Val Ala Asp
                245                 250                 255
Val Lys Ala Asp Leu Ser Ser Ala Leu Ile Arg Leu Thr Glu Ser Gly
            260                 265                 270
Ala Ser Asn Ile Leu Leu Phe Thr Leu Pro Asp Ala Thr Lys Ala Pro
        275                 280                 285
Gln Phe Lys Tyr Ser Thr Gln Glu Glu Ile Glu Thr Val Arg Ala Lys
    290                 295                 300
Ile Leu Glu Phe Asn Thr Phe Ile Glu Glu Gln Ala Leu Leu Tyr Gln
305                 310                 315                 320
Ala Lys Gly Leu Asn Val Ala Leu Tyr Asp Ala His Ser Ile Phe Asp
                325                 330                 335
Gln Leu Thr Ser Asn Pro Lys Gln His Gly Phe Glu Asn Ser Thr Asp
            340                 345                 350
Ala Cys Leu Asn Ile Asn Arg Ser Ser Ser Val Asp Tyr Leu Tyr Ser
        355                 360                 365
His Glu Leu Thr Asn Asp Cys Ala Tyr His Ser Ser Asp Lys Tyr Val
    370                 375                 380
```

Phe Trp Gly Val Thr His Pro Thr Thr Ala Thr His Lys Tyr Ile Ala
385 390 395 400

Asp Gln Ile Ile Gln Thr Lys Leu Asp Gln Phe Asn Phe
405 410

<210> 65
<211> 1164
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 65
atgaaccctt ttcttgaaga taaaattaaa tcctccggtc ccaagaaaat cctcgcctgc 60
gatggcggag gtattttggg tttgatgagc gttgaaatcc tagcaaaaat tgaagcggat 120
ttacgcacta agttaggtaa agaccagaac ttcgtgctcg cggattattt cgattttgtc 180
tgcggcacca gcaccggcgc gattatcgct gcctgtattt ctagtggcat gtcgatggct 240
aaaatacgcc aattctatct cgacagtggg aagcaaatgt tcgataaggc ctccttgctt 300
aagcgcttgc aatacagtta tgacgatgag ccattggcga ggcagttgcg tgcagccttt 360
gatgagcaac tgaaggaaac cgatgccaag ctgggtagtg cgcacctaaa aacgctgttg 420
atgatggtga tgcgtaacca cagcaccgac tcaccttggc cggtttccaa taacccttac 480
gcaaaataca ataatatcgc ccgaaaggat tgcaacctca acctgccttt atggcaattg 540
gtccgtgcca gcaccgccgc tccgacgtat ttcccaccgg aagtcatcac tttcgcagat 600
ggcacacccg aagaatacaa cttcatcttc gtcgacggtg gcgtgaccac ctacaacaac 660
ccagcatatc ttgctttcct aatggccact gccaagcctt atgccctcaa ctggccgaca 720
ggcagcaacc agttattgat cgtttccgta ggcaccggaa gtgccgccaa tgtccgacct 780
aatctggacg tggatgatat gaacctgatc cattttgcca aaaacatccc ttcagccctg 840
atgaatgccg catctgccgg ttgggatatg acctgccggg tattgggtga atgccgccat 900
ggtggcatgt tagatcggga gtttggtgac atggtgatgc ccgcgtcaag agatcttaat 960
tttaccggcc ctaagctttt tacttatatg cgttatgatc ccgatgtttc ctttgagggc 1020
ttgaagacta tcggtatatc agatatcgat ccagccaaaa tgcagcaaat ggattccgtc 1080
aataatattc cagatataca acgggtaggt atcgaatatg ccaaacgcca tgttgataca 1140
gctcattttg aggggtttaa ataa 1164

<210> 66
<211> 387
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 66
Met Asn Pro Phe Leu Glu Asp Lys Ile Lys Ser Ser Gly Pro Lys Lys
1 5 10 15

Ile Leu Ala Cys Asp Gly Gly Gly Ile Leu Gly Leu Met Ser Val Glu
20 25 30

Ile Leu Ala Lys Ile Glu Ala Asp Leu Arg Thr Lys Leu Gly Lys Asp
35 40 45

Gln Asn Phe Val Leu Ala Asp Tyr Phe Asp Phe Val Cys Gly Thr Ser
50 55 60

Thr Gly Ala Ile Ile Ala Ala Cys Ile Ser Ser Gly Met Ser Met Ala
65 70 75 80

Lys Ile Arg Gln Phe Tyr Leu Asp Ser Gly Lys Gln Met Phe Asp Lys
85 90 95

Ala Ser Leu Leu Lys Arg Leu Gln Tyr Ser Tyr Asp Asp Glu Pro Leu

```
                100                 105                 110
Ala Arg Gln Leu Arg Ala Ala Phe Asp Glu Gln Leu Lys Glu Thr Asp
        115                 120                 125
Ala Lys Leu Gly Ser Ala His Leu Lys Thr Leu Leu Met Met Val Met
    130                 135                 140
Arg Asn His Ser Thr Asp Ser Pro Trp Pro Val Ser Asn Asn Pro Tyr
145                 150                 155                 160
Ala Lys Tyr Asn Asn Ile Ala Arg Lys Asp Cys Asn Leu Asn Leu Pro
                165                 170                 175
Leu Trp Gln Leu Val Arg Ala Ser Thr Ala Ala Pro Thr Tyr Phe Pro
            180                 185                 190
Pro Glu Val Ile Thr Phe Ala Asp Gly Thr Pro Glu Glu Tyr Asn Phe
        195                 200                 205
Ile Phe Val Asp Gly Gly Val Thr Thr Tyr Asn Asn Pro Ala Tyr Leu
    210                 215                 220
Ala Phe Leu Met Ala Thr Ala Lys Pro Tyr Ala Leu Asn Trp Pro Thr
225                 230                 235                 240
Gly Ser Asn Gln Leu Leu Ile Val Ser Val Gly Thr Gly Ser Ala Ala
                245                 250                 255
Asn Val Arg Pro Asn Leu Asp Val Asp Asp Met Asn Leu Ile His Phe
            260                 265                 270
Ala Lys Asn Ile Pro Ser Ala Leu Met Asn Ala Ala Ser Ala Gly Trp
        275                 280                 285
Asp Met Thr Cys Arg Val Leu Gly Glu Cys Arg His Gly Gly Met Leu
    290                 295                 300
Asp Arg Glu Phe Gly Asp Met Val Met Pro Ala Ser Arg Asp Leu Asn
305                 310                 315                 320
Phe Thr Gly Pro Lys Leu Phe Thr Tyr Met Arg Tyr Asp Pro Asp Val
                325                 330                 335
Ser Phe Glu Gly Leu Lys Thr Ile Gly Ile Ser Asp Ile Asp Pro Ala
            340                 345                 350
Lys Met Gln Gln Met Asp Ser Val Asn Asn Ile Pro Asp Ile Gln Arg
        355                 360                 365
Val Gly Ile Glu Tyr Ala Lys Arg His Val Asp Thr Ala His Phe Glu
    370                 375                 380
Gly Phe Lys
385
```

<210> 67
<211> 1419
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 67

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atggtcattg tcttcgtcca cggatggagc gtgcgcaaca ccaacacgta cgggcagctg      60
cccttgcgtc tcaagaagag cttcaaagcc gccgggaaac agattcaggt cgagaacatc     120
tacctgggcg agtacgtgag ctttgacgac caggtaacag tcgacgacat cgcccgcgca     180
ttcgattgcg cactgcggga aaaactatac gatccggcga cgaagcagtg gacgaagttc     240
gcctgcatca ctcattccac cggcggcccg gtcgcgcgct tgtggatgga tctctactac     300
ggcgccgcca gactggccga gtgcccgatg tcccacctcg tgatgctcgc cccggccaat     360
catggctcgg cccttgccca gctcggcaag agccgcctca gccgcatcaa gagcttcttc     420
gagggtgtcg aaccgggcca gcgcgtcctc gactggctcg aactcggcag tgagctgagt     480
tgggccctca acacgagatg gctcgactac gactgccgcg ccgccgcctg ctgggtcttc     540
accctcaccg gccagcgcat cgaccggagt ttgtacgacc atctcaacag ctataccggt     600
```

gagcagggat cggatggcgt cgtgcgcgtc gccgcggcca acatgaacac caagctgctg 660
acctttgaac agaaggggcg caagctcgtg ttcacaggcc agaagaagac cgccgacacc 720
ggccttggcg tcgtgccggg ccggtcgcac tccggccgcg acatgggcat catcgccagc 780
gtgcgcggca ccggcgacca tcccaccctg gaatgggtga ctcgttgcct ggccgtcacc 840
gacgtcaaca cgtacgatgc cgtctgtaag gatctggacg ctctcaccgc ccagacccag 900
aaggatgaaa aggtggaaga ggtcaaaggc ctgctgcgga cggtcagata ccagacggac 960
cgctacgtca tgctcgtctt ccgcctgaag aacgaccgcg gcgactacct ctccgattac 1020
gatctcctgc tcaccgccgg acccaactac tcgcccgacg acctgcccga aggcttcttc 1080
gtcgaccgcc aacggaacca gcggaacccg ggcaagctca cttactacct gaactacgac 1140
gccatggcca aattgaaagg taagaccgcc gagggccgtc tgggcttcaa gatcctggcg 1200
cgcccggtga aaggcggcct cgtctactat gaggttgcgg agttccagtc cgacgtgggc 1260
ggcgtcagca gcatgctgca gcccaacgca acagtgatga tcgacatcac cctcaatcgc 1320
aacgtcgacg cgcgcgtctt ccggttcacc gagaatctgc ccacgggtga ccagggcgag 1380
gaaatcagcg gcgtcccgct ggggcagaac gtcccgtag 1419

<210> 68
<211> 472
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 68
Met Val Ile Val Phe Val His Gly Trp Ser Val Arg Asn Thr Asn Thr
1 5 10 15
Tyr Gly Gln Leu Pro Leu Arg Leu Lys Lys Ser Phe Lys Ala Ala Gly
20 25 30
Lys Gln Ile Gln Val Glu Asn Ile Tyr Leu Gly Glu Tyr Val Ser Phe
35 40 45
Asp Asp Gln Val Thr Val Asp Asp Ile Ala Arg Ala Phe Asp Cys Ala
50 55 60
Leu Arg Glu Lys Leu Tyr Asp Pro Ala Thr Lys Gln Trp Thr Lys Phe
65 70 75 80
Ala Cys Ile Thr His Ser Thr Gly Gly Pro Val Ala Arg Leu Trp Met
85 90 95
Asp Leu Tyr Tyr Gly Ala Ala Arg Leu Ala Glu Cys Pro Met Ser His
100 105 110
Leu Val Met Leu Ala Pro Ala Asn His Gly Ser Ala Leu Ala Gln Leu
115 120 125
Gly Lys Ser Arg Leu Ser Arg Ile Lys Ser Phe Phe Glu Gly Val Glu
130 135 140
Pro Gly Gln Arg Val Leu Asp Trp Leu Glu Leu Gly Ser Glu Leu Ser
145 150 155 160
Trp Ala Leu Asn Thr Arg Trp Leu Asp Tyr Asp Cys Arg Ala Ala Ala
165 170 175
Cys Trp Val Phe Thr Leu Thr Gly Gln Arg Ile Asp Arg Ser Leu Tyr
180 185 190
Asp His Leu Asn Ser Tyr Thr Gly Glu Gln Gly Ser Asp Gly Val Val
195 200 205
Arg Val Ala Ala Ala Asn Met Asn Thr Lys Leu Leu Thr Phe Glu Gln
210 215 220
Lys Gly Arg Lys Leu Val Phe Thr Gly Gln Lys Lys Thr Ala Asp Thr
225 230 235 240
Gly Leu Gly Val Val Pro Gly Arg Ser His Ser Gly Arg Asp Met Gly
245 250 255
Ile Ile Ala Ser Val Arg Gly Thr Gly Asp His Pro Thr Leu Glu Trp

```
                260                 265                 270
Val Thr Arg Cys Leu Ala Val Thr Asp Val Asn Thr Tyr Asp Ala Val
        275                 280                 285
Cys Lys Asp Leu Asp Ala Leu Thr Ala Gln Thr Gln Lys Asp Glu Lys
    290                 295                 300
Val Glu Glu Val Lys Gly Leu Leu Arg Thr Val Arg Tyr Gln Thr Asp
305                 310                 315                 320
Arg Tyr Val Met Leu Val Phe Arg Leu Lys Asn Asp Arg Gly Asp Tyr
                325                 330                 335
Leu Ser Asp Tyr Asp Leu Leu Leu Thr Ala Gly Pro Asn Tyr Ser Pro
            340                 345                 350
Asp Asp Leu Pro Glu Gly Phe Phe Val Asp Arg Gln Arg Asn Gln Arg
        355                 360                 365
Asn Pro Gly Lys Leu Thr Tyr Tyr Leu Asn Tyr Asp Ala Met Ala Lys
    370                 375                 380
Leu Lys Gly Lys Thr Ala Glu Gly Arg Leu Gly Phe Lys Ile Leu Ala
385                 390                 395                 400
Arg Pro Val Lys Gly Gly Leu Val Tyr Tyr Glu Val Ala Glu Phe Gln
                405                 410                 415
Ser Asp Val Gly Gly Val Ser Ser Met Leu Gln Pro Asn Ala Thr Val
            420                 425                 430
Met Ile Asp Ile Thr Leu Asn Arg Asn Val Asp Ala Arg Val Phe Arg
        435                 440                 445
Phe Thr Glu Asn Leu Pro Thr Gly Asp Gln Gly Glu Glu Ile Ser Gly
    450                 455                 460
Val Pro Leu Gly Gln Asn Val Pro
465                 470
```

<210> 69
<211> 1038
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 69

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atgacaacac aatttagaaa cttgatattt gaaggcggcg gtgtaaaagg tgttgcttac      60
attggcgcca tgcagattct cgaaaatcgt ggcgtgttgc aagatattcg ccgagtcgga     120
gggtgcagtg cgggtgcgat caacgcgctg atttttgcgc tgggttacac tgtccgtgag     180
caaaaagaga tcttacaagc cacggatttt aaccagttta tggataactc ttggggtgtt     240
attcgtgata ttcgcaggct tgctcgagac tttggctggc acaagggtga cttctttaat     300
agctggatag gtgatttgat tcatcgtcgt ttggggaatc gccgagcgac gttcaaagat     360
ctgcaaaagg ccaagcttcc tgatctttat gtcatcggta ctaatctgtc tacggggtat     420
gcagaggttt tttcagccga aagacacccc gatatggagc tagcgacagc ggtgcgtatc     480
tccatgtcga taccgctgtt ctttgcggcc gtgcgccacg gtgaccgaca agatgtgtat     540
gtcgatgggg gtgttcaact taactatccg attaaacttt ttgatcggga gcgttacatt     600
gatctggcca aagatcccgg tgccgttcgg cgaacgggct attacaacaa agaaaacgct     660
cgctttcagc ttgagcggcc gggctatagc cctatgttt acaatcgcca gaccttgggt      720
ttgcgactag atagtcgaga ggagataggg ctctttcgtt atgacgaacc cctcaagggc     780
aaacccatta agtccttcac tgactacgct cgacaacttt tcggtgcgtt gatgaatgca     840
caggaaaaga ttcatctaca tggcgatgat tggcagcgca cggtctatat cgatacattg     900
gatgtgggta cgacggactt caatctttct gatgcaacta agcaagcact gattgaacag     960
ggaattaacg gcaccgaaaa ttatttcgag tggtttgata atccgttgga gaagcctgtt    1020
aatagagtgg agtcatag                                                  1038
```

<210> 70

<211> 345
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 70
Met Thr Thr Gln Phe Arg Asn Leu Ile Phe Glu Gly Gly Gly Val Lys
1 5 10 15
Gly Val Ala Tyr Ile Gly Ala Met Gln Ile Leu Glu Asn Arg Gly Val
20 25 30
Leu Gln Asp Ile Arg Arg Val Gly Gly Cys Ser Ala Gly Ala Ile Asn
35 40 45
Ala Leu Ile Phe Ala Leu Gly Tyr Thr Val Arg Glu Gln Lys Glu Ile
50 55 60
Leu Gln Ala Thr Asp Phe Asn Gln Phe Met Asp Asn Ser Trp Gly Val
65 70 75 80
Ile Arg Asp Ile Arg Arg Leu Ala Arg Asp Phe Gly Trp His Lys Gly
85 90 95
Asp Phe Phe Asn Ser Trp Ile Gly Asp Leu Ile His Arg Arg Leu Gly
100 105 110
Asn Arg Arg Ala Thr Phe Lys Asp Leu Gln Lys Ala Lys Leu Pro Asp
115 120 125
Leu Tyr Val Ile Gly Thr Asn Leu Ser Thr Gly Tyr Ala Glu Val Phe
130 135 140
Ser Ala Glu Arg His Pro Asp Met Glu Leu Ala Thr Ala Val Arg Ile
145 150 155 160
Ser Met Ser Ile Pro Leu Phe Phe Ala Ala Val Arg His Gly Asp Arg
165 170 175
Gln Asp Val Tyr Val Asp Gly Gly Val Gln Leu Asn Tyr Pro Ile Lys
180 185 190
Leu Phe Asp Arg Glu Arg Tyr Ile Asp Leu Ala Lys Asp Pro Gly Ala
195 200 205
Val Arg Arg Thr Gly Tyr Tyr Asn Lys Glu Asn Ala Arg Phe Gln Leu
210 215 220
Glu Arg Pro Gly Tyr Ser Pro Tyr Val Tyr Asn Arg Gln Thr Leu Gly
225 230 235 240
Leu Arg Leu Asp Ser Arg Glu Glu Ile Gly Leu Phe Arg Tyr Asp Glu
245 250 255
Pro Leu Lys Gly Lys Pro Ile Lys Ser Phe Thr Asp Tyr Ala Arg Gln
260 265 270
Leu Phe Gly Ala Leu Met Asn Ala Gln Glu Lys Ile His Leu His Gly
275 280 285
Asp Asp Trp Gln Arg Thr Val Tyr Ile Asp Thr Leu Asp Val Gly Thr
290 295 300
Thr Asp Phe Asn Leu Ser Asp Ala Thr Lys Gln Ala Leu Ile Glu Gln
305 310 315 320
Gly Ile Asn Gly Thr Glu Asn Tyr Phe Glu Trp Phe Asp Asn Pro Leu
325 330 335
Glu Lys Pro Val Asn Arg Val Glu Ser
340 345

<210> 71
<211> 3264
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 71
atgtcgctat catcaccgcc cgaaaccccc gaaccccccg aaccccccgtc acccggcgcg 60
cgatcgctcc ggggaggatg gagccgccgg gtggccggcc tgctggccct ggtgctgctc 120
accgggctcc tccagatcgt cgtgccgctc gcacggcccg ccgcggcggc cgtacagcag 180
cccgcgatga cgtggaacct gcatggggcc aagaagaccg cggaactggt tcccgatctg 240
atgcgtaacc ataacgtcac cgtcgcggcc ctccaggaag tggccaacgg caacttcctg 300
ggcctcactc ccacagagca cgacgtgccc tacctcaagc cggacggcac gacctcgact 360
ccgccggatc cgcagaaatg gcgggtcgag aagtacaacc tcgccaagga cgatgcaacc 420
gctttcgtga tccggaccgg ctccaacaac cgcgggctcg cgatcgtcac cacccaggac 480
gtcggcgatg tctcgcagaa tgtacacgtc gtcaatgtga ccgaggattg ggaaggcaag 540
atgttccccg ccctgggggt gaagatcgac ggcgcctggt actactccat ccacgcctcc 600
accacgccga agcgcgcgaa caacaacgcc ggcactctgg tcgaggacct ctccaagctg 660
cacgagacgg ccgctttcga aggcgactgg gccgcgatgg gcgactggaa ccggtacccc 720
tccgaggact cgaacgccta cgagaaccaa cggaagcatc tcaaaggcgc catgcggaca 780
aactttccgg ataatcaggc ggcgttgcgc gaagtcctgg agttcgagtc cgacgaacgc 840
gtcatctggc agggtgcgag gacccacgac cacggcgccg agctcgacta catggtggcc 900
aagggagccg gtaacgacta caaggccagc cgatcgacgt cgaagcacgg ctccgatcac 960
tacccggtgt tcttcggtat tggggacgat tcggacacct gcatgggcgg cacggcgccg 1020
gtggcggcga acgcgccgcg tgcggccgcc accgagtcct gtcccctgga cgacgatctg 1080
ccggccgtca tcgtctcgat gggggacagc tatatctccg gcgagggagg gcgctggcag 1140
ggcaacgcca acacctcctc cgggggcgac tcctggggca ccgaccgggc cgccgacggc 1200
acggaggtct acgagaagaa ctccgaaggc agcgatgcct gtcaccgctc cgacgtcgcg 1260
gagatcaagc gcgccgacat cgccgacatc ccggcggaac gcaggatcaa catcgcctgc 1320
tcgggcgccg agaccaagca cctgctcacc gagaccttca agggtgaaaa gccccagatc 1380
gagcagctcg ccgacgtcgc cgaaacccac cgggtggaca cgatcgtggt ctccatcggc 1440
ggcaacgacc tcgagttcgc cgacatcgtg agccagtgcg ccacggcctt catgctcggg 1500
gaaggcgcgt gtcacacgga cgtcgacgat acccttgata gccggttggg cgatgtgagc 1560
agatccgtct ccgaggttct ggccgccatc cgcgacacca tgatcgaggc cgggcaggac 1620
gataccagct acaagctcgt tctccagtcc taccctgccc cgttgcccgc gtcggatgag 1680
atgcggtaca cgggcgatca ctacgaccgg tacaccgagg gcggctgccc cttctatgac 1740
gtcgacctgg actggacgcg cgacgtcctc atcaaaaaga tcgaagccac gctgcgcggg 1800
gtggccaaga gtgcggatgc ggccttcctc aacctgacgg acacgttcac ggggcacgag 1860
ctgtgctcga agcacacccg acaggcggag tccggcgaat cgctggcgaa tccaatactg 1920
gaacacgagg ccgagtgggt gcgcttcgta ccaggtctca ccacgccggg tgacacggcc 1980
gaagccatcc atccgaatgc gttcggccag cacgccctca gtagctgcct cagccaggcc 2040
gtccggacga tggacgattc ggaccagagg tacttcgagt gcgacgggcg ggacaccgga 2100
aatccccgcc tcgtgtggcc acgcagttcg cccatcgacg ccgtcgtgga gaccgcggac 2160
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gcccgcttca acccggacgc ggaccggagc ggcgcgatcg atccgggccg aatcaccttc 2280
ggccaaaccg acggatggct cggtgaggtg aaggacactt cgaactggcc gagcctgagt 2340
ggaaccgact tcgtcgacgg catcgacgcc gccgccgagg cacgcaccag caccggtcac 2400
cagctgctgc tgttccacag cggcgttgag gacaaccagt acgtgcgggt cgagatggcg 2460
ccgggcacca ctgacgacca gctcgtcagg ggccccgtgc ccatcacgag gtactggccc 2520
ctcttccagg acacccccttt cgaatggggc gtggatgccg ccgcggggga ccagctgaac 2580
cgggcgatgg tcttcaggca cggctatgtg gggctggtgc aggtctccct cgacgctctc 2640
agcgacgaat ggctcgtgga accgacgttg atcggctcgg cgattccggc gctggagggc 2700
accccgttcg agacaggggt ggacgcggcg atcgtgcggc accagcaacc gacggccatg 2760
tgggtcgacc tgatcagcgg tacgcaggtg gtgacgctgc tggtggactt ggacgatctg 2820
tcgaagagca cgtacatgac gagcatcgtg gagatcacga cgatgtggcc gagcctgcgc 2880
ggcagcatct tcgactggac cggcggagag gcgtggaagc cggagaagat gcagatcaag 2940
accggcgcgg gcgatcccta cgacatggac gccgacgacc ggcaggccaa gcctgcggtg 3000
tcgggctcgc acgagcagtg ccgtccggag ggactagcgc agaccccccgg cgtgaacacg 3060
ccgtactgcg aggtgtacga caccgacggc cgcgaatggc tgggcgggaa cgggcacgac 3120

aggcgggtca tcggctactt caccggctgg cgcaccggtg agaacgacca gccgcgctac 3180
ctggtgccga acatcccgtg gtcgaaggtg acccacatca actacgcgtt cgcgaaagtc 3240
gacgacgaca acaagatcca aaga 3264

<210> 72
<211> 1088
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 72
Met Ser Leu Ser Ser Pro Pro Glu Thr Pro Glu Pro Pro Glu Pro Pro
1 5 10 15
Ser Pro Gly Ala Arg Ser Leu Arg Gly Gly Trp Ser Arg Arg Val Ala
20 25 30
Gly Leu Leu Ala Leu Val Leu Leu Thr Gly Leu Leu Gln Ile Val Val
35 40 45
Pro Leu Ala Arg Pro Ala Ala Ala Ala Val Gln Gln Pro Ala Met Thr
50 55 60
Trp Asn Leu His Gly Ala Lys Lys Thr Ala Glu Leu Val Pro Asp Leu
65 70 75 80
Met Arg Asn His Asn Val Thr Val Ala Ala Leu Gln Glu Val Ala Asn
85 90 95
Gly Asn Phe Leu Gly Leu Thr Pro Thr Glu His Asp Val Pro Tyr Leu
100 105 110
Lys Pro Asp Gly Thr Thr Ser Thr Pro Pro Asp Pro Gln Lys Trp Arg
115 120 125
Val Glu Lys Tyr Asn Leu Ala Lys Asp Asp Ala Thr Ala Phe Val Ile
130 135 140
Arg Thr Gly Ser Asn Asn Arg Gly Leu Ala Ile Val Thr Thr Gln Asp
145 150 155 160
Val Gly Asp Val Ser Gln Asn Val His Val Val Asn Val Thr Glu Asp
165 170 175
Trp Glu Gly Lys Met Phe Pro Ala Leu Gly Val Lys Ile Asp Gly Ala
180 185 190
Trp Tyr Tyr Ser Ile His Ala Ser Thr Thr Pro Lys Arg Ala Asn Asn
195 200 205
Asn Ala Gly Thr Leu Val Glu Asp Leu Ser Lys Leu His Glu Thr Ala
210 215 220
Ala Phe Glu Gly Asp Trp Ala Ala Met Gly Asp Trp Asn Arg Tyr Pro
225 230 235 240
Ser Glu Asp Ser Asn Ala Tyr Glu Asn Gln Arg Lys His Leu Lys Gly
245 250 255
Ala Met Arg Thr Asn Phe Pro Asp Asn Gln Ala Ala Leu Arg Glu Val
260 265 270
Leu Glu Phe Glu Ser Asp Glu Arg Val Ile Trp Gln Gly Ala Arg Thr
275 280 285
His Asp His Gly Ala Glu Leu Asp Tyr Met Val Ala Lys Gly Ala Gly
290 295 300
Asn Asp Tyr Lys Ala Ser Arg Ser Thr Ser Lys His Gly Ser Asp His
305 310 315 320
Tyr Pro Val Phe Phe Gly Ile Gly Asp Asp Ser Asp Thr Cys Met Gly
325 330 335
Gly Thr Ala Pro Val Ala Ala Asn Ala Pro Arg Ala Ala Ala Thr Glu
340 345 350

```
Ser Cys Pro Leu Asp Asp Asp Leu Pro Ala Val Ile Val Ser Met Gly
        355                 360                 365
Asp Ser Tyr Ile Ser Gly Glu Gly Gly Arg Trp Gln Gly Asn Ala Asn
    370                 375                 380
Thr Ser Ser Gly Gly Asp Ser Trp Gly Thr Asp Arg Ala Ala Asp Gly
385                 390                 395                 400
Thr Glu Val Tyr Glu Lys Asn Ser Glu Gly Ser Asp Ala Cys His Arg
                405                 410                 415
Ser Asp Val Ala Glu Ile Lys Arg Ala Asp Ile Ala Asp Ile Pro Ala
            420                 425                 430
Glu Arg Arg Ile Asn Ile Ala Cys Ser Gly Ala Glu Thr Lys His Leu
        435                 440                 445
Leu Thr Glu Thr Phe Lys Gly Glu Lys Pro Gln Ile Glu Gln Leu Ala
    450                 455                 460
Asp Val Ala Glu Thr His Arg Val Asp Thr Ile Val Val Ser Ile Gly
465                 470                 475                 480
Gly Asn Asp Leu Glu Phe Ala Asp Ile Val Ser Gln Cys Ala Thr Ala
                485                 490                 495
Phe Met Leu Gly Glu Gly Ala Cys His Thr Asp Val Asp Asp Thr Leu
            500                 505                 510
Asp Ser Arg Leu Gly Asp Val Ser Arg Ser Val Ser Glu Val Leu Ala
        515                 520                 525
Ala Ile Arg Asp Thr Met Ile Glu Ala Gly Gln Asp Asp Thr Ser Tyr
    530                 535                 540
Lys Leu Val Leu Gln Ser Tyr Pro Ala Pro Leu Pro Ala Ser Asp Glu
545                 550                 555                 560
Met Arg Tyr Thr Gly Asp His Tyr Asp Arg Tyr Thr Glu Gly Gly Cys
                565                 570                 575
Pro Phe Tyr Asp Val Asp Leu Asp Trp Thr Arg Asp Val Leu Ile Lys
            580                 585                 590
Lys Ile Glu Ala Thr Leu Arg Gly Val Ala Lys Ser Ala Asp Ala Ala
        595                 600                 605
Phe Leu Asn Leu Thr Asp Thr Phe Thr Gly His Glu Leu Cys Ser Lys
    610                 615                 620
His Thr Arg Gln Ala Glu Ser Gly Glu Ser Leu Ala Asn Pro Ile Leu
625                 630                 635                 640
Glu His Glu Ala Glu Trp Val Arg Phe Val Pro Gly Leu Thr Thr Pro
                645                 650                 655
Gly Asp Thr Ala Glu Ala Ile His Pro Asn Ala Phe Gly Gln His Ala
            660                 665                 670
Leu Ser Ser Cys Leu Ser Gln Ala Val Arg Thr Met Asp Asp Ser Asp
        675                 680                 685
Gln Arg Tyr Phe Glu Cys Asp Gly Arg Asp Thr Gly Asn Pro Arg Leu
    690                 695                 700
Val Trp Pro Arg Ser Ser Pro Ile Asp Ala Val Val Glu Thr Ala Asp
705                 710                 715                 720
Gly Trp Gln Gly Asp Asp Phe Arg Leu Ala Asp His Tyr Met Phe Gln
                725                 730                 735
Arg Gly Val Tyr Ala Arg Phe Asn Pro Asp Ala Asp Arg Ser Gly Ala
            740                 745                 750
Ile Asp Pro Gly Arg Ile Thr Phe Gly Gln Thr Asp Gly Trp Leu Gly
        755                 760                 765
Glu Val Lys Asp Thr Ser Asn Trp Pro Ser Leu Ser Gly Thr Asp Phe
    770                 775                 780
Val Asp Gly Ile Asp Ala Ala Ala Glu Ala Arg Thr Ser Thr Gly His
785                 790                 795                 800
Gln Leu Leu Leu Phe His Ser Gly Val Glu Asp Asn Gln Tyr Val Arg
```

```
                805                 810                 815
Val Glu Met Ala Pro Gly Thr Thr Asp Asp Gln Leu Val Arg Gly Pro
            820                 825                 830
Val Pro Ile Thr Arg Tyr Trp Pro Leu Phe Gln Asp Thr Pro Phe Glu
        835                 840                 845
Trp Gly Val Asp Ala Ala Ala Gly Asp Gln Leu Asn Arg Ala Met Val
    850                 855                 860
Phe Arg His Gly Tyr Val Gly Leu Val Gln Val Ser Leu Asp Ala Leu
865                 870                 875                 880
Ser Asp Glu Trp Leu Val Glu Pro Thr Leu Ile Gly Ser Ala Ile Pro
                885                 890                 895
Ala Leu Glu Gly Thr Pro Phe Glu Thr Gly Val Asp Ala Ala Ile Val
            900                 905                 910
Arg His Gln Gln Pro Thr Ala Met Trp Val Asp Leu Ile Ser Gly Thr
        915                 920                 925
Gln Val Val Thr Leu Leu Val Asp Leu Asp Asp Leu Ser Lys Ser Thr
    930                 935                 940
Tyr Met Thr Ser Ile Val Glu Ile Thr Thr Met Trp Pro Ser Leu Arg
945                 950                 955                 960
Gly Ser Ile Phe Asp Trp Thr Gly Gly Glu Ala Trp Lys Pro Glu Lys
                965                 970                 975
Met Gln Ile Lys Thr Gly Ala Gly Asp Pro Tyr Asp Met Asp Ala Asp
            980                 985                 990
Asp Arg Gln Ala Lys Pro Ala Val Ser Gly Ser His Glu Gln Cys Arg
        995                 1000                1005
Pro Glu Gly Leu Ala Gln Thr Pro Gly Val Asn Thr Pro Tyr Cys Glu
    1010                1015                1020
Val Tyr Asp Thr Asp Gly Arg Glu Trp Leu Gly Gly Asn Gly His Asp
1025                1030                1035                1040
Arg Arg Val Ile Gly Tyr Phe Thr Gly Trp Arg Thr Gly Glu Asn Asp
                1045                1050                1055
Gln Pro Arg Tyr Leu Val Pro Asn Ile Pro Trp Ser Lys Val Thr His
            1060                1065                1070
Ile Asn Tyr Ala Phe Ala Lys Val Asp Asp Asp Asn Lys Ile Gln Arg
        1075                1080                1085
```

<210> 73
<211> 753
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 73
```
atgggaaacg gtgcagcagt tggttccaat gataatggta gagaagaaag tgtttacgta     60
ctttctgtga tcgcctgtaa tgtttattat ttacagaagt gtgaaggtgg ggcatcgcgt    120
gatagcgtga ttagagaaat taatagccaa actcaacctt taggatatga gattgtagca    180
gattctattc gtgatggtca tattggttct tttgcctgta agatggcagt ctttagaaat    240
aatggtaatg gcaattgtgt tttagcgatc aaagggacag atatgaataa tatcaatgac    300
ttggtgaatg atctaaccat gatattagga ggcattggtt ctgttgctgc aatccaacca    360
acgattaaca tggcacaaga actcatcgac caatatggag tgaatttgat tactggtcac    420
tcccttggag gctacatgac tgaaatcatc gctaccaatc gtggactacc aggtattgca    480
ttttgcgcac caggttcaaa tggtccaatt gtaaaattag gtggacaaga gacacctggc    540
tttcacaatg ttaactttga acatgatcca gcaggtaacg ttatgactgg ggtttatact    600
catgtccaat ggagtattta tgtaggatgt gatggtatga ctcatggtat tgaaaatatg    660
gtgaattatt ttaaagataa aagagattta accaatcgca atattcaagg aagaagtgaa    720
```

agtcataata cgggttatta ttacccaaaa taa 753

<210> 74
<211> 250
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 74
Met Gly Asn Gly Ala Ala Val Gly Ser Asn Asp Asn Gly Arg Glu Glu
1 5 10 15
Ser Val Tyr Val Leu Ser Val Ile Ala Cys Asn Val Tyr Tyr Leu Gln
20 25 30
Lys Cys Glu Gly Gly Ala Ser Arg Asp Ser Val Ile Arg Glu Ile Asn
35 40 45
Ser Gln Thr Gln Pro Leu Gly Tyr Glu Ile Val Ala Asp Ser Ile Arg
50 55 60
Asp Gly His Ile Gly Ser Phe Ala Cys Lys Met Ala Val Phe Arg Asn
65 70 75 80
Asn Gly Asn Gly Asn Cys Val Leu Ala Ile Lys Gly Thr Asp Met Asn
85 90 95
Asn Ile Asn Asp Leu Val Asn Asp Leu Thr Met Ile Leu Gly Gly Ile
100 105 110
Gly Ser Val Ala Ala Ile Gln Pro Thr Ile Asn Met Ala Gln Glu Leu
115 120 125
Ile Asp Gln Tyr Gly Val Asn Leu Ile Thr Gly His Ser Leu Gly Gly
130 135 140
Tyr Met Thr Glu Ile Ile Ala Thr Asn Arg Gly Leu Pro Gly Ile Ala
145 150 155 160
Phe Cys Ala Pro Gly Ser Asn Gly Pro Ile Val Lys Leu Gly Gly Gln
165 170 175
Glu Thr Pro Gly Phe His Asn Val Asn Phe Glu His Asp Pro Ala Gly
180 185 190
Asn Val Met Thr Gly Val Tyr Thr His Val Gln Trp Ser Ile Tyr Val
195 200 205
Gly Cys Asp Gly Met Thr His Gly Ile Glu Asn Met Val Asn Tyr Phe
210 215 220
Lys Asp Lys Arg Asp Leu Thr Asn Arg Asn Ile Gln Gly Arg Ser Glu
225 230 235 240
Ser His Asn Thr Gly Tyr Tyr Tyr Pro Lys
245 250

<210> 75
<211> 1335
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 75
atgactacta aaatcttttt aattcacgga tggtctgtca agacaacaca aacatatcag 60
gcgctgcacc ttaagttggc agagcaggga tatcagctgg aagatattta cctcgggcgg 120
tatctgtccc ttgaaaatca tatcgaaata cgggatattg caaaagcaat gcaccgtgca 180
ttgctggaga ggattaccga ctggagtcag cctttccatt ttattactca cagtacggga 240

ggtatggtcg ccaaatattg gatattgaat cattataaag gaagtattgc aaaacaaaaa 300
ccactcaaaa atgtagtgtt tctggctgca cctaattttg gttcaaggct ggcacaccat 360
ggacgtacca tgctgggaga aataatggaa ctgggagaaa cagggaagaa gattcttgaa 420
tctctggagt taggaagtgc tttttcgtgg gatgtgaatg agcagttttt taatgcgtcc 480
aattggaaag ataaagaaat aaagttctat aacctgatag gagacagggt caaaacggat 540
tttttttaaat ccaaaatttt tccagctgcg tttgaaagcg ggtcagatat ggtgattcgg 600
gttgcggcag gaaatcagaa ctttgtccgg tacaggtacg atagtcagaa agatagcttt 660
actgttgtca atgagttgaa aggaattgct tttggtgctc tctaccaata tacacattcc 720
aatgatgatt atggaatcct gaacagcatc aaaaaaagtt caacccttga aaaccatcag 780
gcactcagac taattgtaga atgtctgaag gtttcgggag ataaagaata tgaaaatgtt 840
gttgcacagt tggctgcagc gacaaaagaa accagagaaa aacgccaggg atatgcacag 900
ctggatttcc gttttcggga tgatgaaggc tttccaatag atgattatgt tgtagagctg 960
ggagtaatgg taaatggaaa acctaaacca tctaaaacag tagatgacgt gcataagaat 1020
aaaattacac caaaccatct tactgtattc attaacctga aagaactgga acctaatctg 1080
aagtacttta tcaatattaa atcgatatcg gaatcctcca tgtatagtta cgatcctgct 1140
gtcaggacta tagagcttgc ttctaacgag attacaaaaa ttatccgtga ggaccataca 1200
acacagattg atgtgatact ttcccggact cctgctaaaa accttttcat gtttcatcgc 1260
ggagatgatg aagacctaca tgtgacatgg tcgcggtacg gagaaacaaa aagtacaaag 1320
cagggaataa aataa 1335

<210> 76
<211> 444
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 76
Met Thr Thr Lys Ile Phe Leu Ile His Gly Trp Ser Val Lys Thr Thr
1 5 10 15
Gln Thr Tyr Gln Ala Leu His Leu Lys Leu Ala Glu Gln Gly Tyr Gln
20 25 30
Leu Glu Asp Ile Tyr Leu Gly Arg Tyr Leu Ser Leu Glu Asn His Ile
35 40 45
Glu Ile Arg Asp Ile Ala Lys Ala Met His Arg Ala Leu Leu Glu Arg
50 55 60
Ile Thr Asp Trp Ser Gln Pro Phe His Phe Ile Thr His Ser Thr Gly
65 70 75 80
Gly Met Val Ala Lys Tyr Trp Ile Leu Asn His Tyr Lys Gly Ser Ile
85 90 95
Ala Lys Gln Lys Pro Leu Lys Asn Val Val Phe Leu Ala Ala Pro Asn
100 105 110
Phe Gly Ser Arg Leu Ala His His Gly Arg Thr Met Leu Gly Glu Ile
115 120 125
Met Glu Leu Gly Glu Thr Gly Lys Lys Ile Leu Glu Ser Leu Glu Leu
130 135 140
Gly Ser Ala Phe Ser Trp Asp Val Asn Glu Gln Phe Phe Asn Ala Ser
145 150 155 160
Asn Trp Lys Asp Lys Glu Ile Lys Phe Tyr Asn Leu Ile Gly Asp Arg
165 170 175
Val Lys Thr Asp Phe Phe Lys Ser Lys Ile Phe Pro Ala Ala Phe Glu
180 185 190
Ser Gly Ser Asp Met Val Ile Arg Val Ala Ala Gly Asn Gln Asn Phe
195 200 205
Val Arg Tyr Arg Tyr Asp Ser Gln Lys Asp Ser Phe Thr Val Val Asn
210 215 220

Glu Leu Lys Gly Ile Ala Phe Gly Ala Leu Tyr Gln Tyr Thr His Ser
225 230 235 240
Asn Asp Asp Tyr Gly Ile Leu Asn Ser Ile Lys Lys Ser Ser Thr Leu
245 250 255
Glu Asn His Gln Ala Leu Arg Leu Ile Val Glu Cys Leu Lys Val Ser
260 265 270
Gly Asp Lys Glu Tyr Glu Asn Val Val Ala Gln Leu Ala Ala Ala Thr
275 280 285
Lys Glu Thr Arg Glu Lys Arg Gln Gly Tyr Ala Gln Leu Asp Phe Arg
290 295 300
Phe Arg Asp Asp Glu Gly Phe Pro Ile Asp Asp Tyr Val Val Glu Leu
305 310 315 320
Gly Val Met Val Asn Gly Lys Pro Lys Pro Ser Lys Thr Val Asp Asp
325 330 335
Val His Lys Asn Lys Ile Thr Pro Asn His Leu Thr Val Phe Ile Asn
340 345 350
Leu Lys Glu Leu Glu Pro Asn Leu Lys Tyr Phe Ile Asn Ile Lys Ser
355 360 365
Ile Ser Glu Ser Ser Met Tyr Ser Tyr Asp Pro Ala Val Arg Thr Ile
370 375 380
Glu Leu Ala Ser Asn Glu Ile Thr Lys Ile Ile Arg Glu Asp His Thr
385 390 395 400
Thr Gln Ile Asp Val Ile Leu Ser Arg Thr Pro Ala Lys Asn Leu Phe
405 410 415
Met Phe His Arg Gly Asp Asp Glu Asp Leu His Val Thr Trp Ser Arg
420 425 430
Tyr Gly Glu Thr Lys Ser Thr Lys Gln Gly Ile Lys
435 440

<210> 77
<211> 1026
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 77
atggcttatc actttaaaaa cttggtcttc gaaggcggtg gcgtgaaagg catcgcctac 60
gtgggtgctc ttgaagtact tgagagagaa ggcattctga aagacatcaa acgcgtggct 120
ggtacttcgg ctggagcgct ggttgccgtc ttaatcagtt tgggctatac cgcccaagaa 180
ttgaaggaca tcctatggaa aatcaatttc caaaactttt tggacagctc gtggggcttg 240
gtgcgcaaca cggcacgttt cattgaggat tacggttggt acaaaggtga gtttttccgc 300
gaattggttg ccggctacat caaggaaaaa acgggcaata gtgaaagcac tttcaaggat 360
ctggccaaat caaaagattt ccgtggcctc agccttattg gtagcgatct gtccacagga 420
tactcaaagg tgttcagcaa cgaattcacc ccaaacgtca aagtagctga tgcagcccgc 480
atctccatgt cgatacccct gtttttcaaa gccgttcgcg gtgtaaacgg tgatggacac 540
atttacgtcg atggtggact gttagacaac tatgccatca aggtgttcga ccgcgtcaat 600
tacgtaaaga ataagaacaa cgtacggtac accgagtatt atgaaaagac caacaagtcg 660
ctgaaaagca aaaacaagct gaccaacgaa tacgtctaca ataaagaaac tttgggcttc 720
cgattggatg ccaaagaaca gattgagatg tttctcgacc atagtataga accaaaggca 780
aaggacattg actcactatt ctcttacacg aaggctttgg tcaccaccct catcgacttt 840
caaaacaatg tacatttgca tagtgacgac tggcaacgca cagtctatat cgactcttta 900
ggtatcagtt ccactgactt cggcatctct gactctaaaa aacagaaact cgtcgattca 960
ggcattttgc atacgcaaaa atacctggat tggtataaca acgacgaaga gaaagccaac 1020
aaatag 1026

```
<210> 78
<211> 341
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 78
Met Ala Tyr His Phe Lys Asn Leu Val Phe Glu Gly Gly Gly Val Lys
 1               5                  10                  15
Gly Ile Ala Tyr Val Gly Ala Leu Glu Val Leu Glu Arg Glu Gly Ile
            20                  25                  30
Leu Lys Asp Ile Lys Arg Val Ala Gly Thr Ser Ala Gly Ala Leu Val
        35                  40                  45
Ala Val Leu Ile Ser Leu Gly Tyr Thr Ala Gln Glu Leu Lys Asp Ile
    50                  55                  60
Leu Trp Lys Ile Asn Phe Gln Asn Phe Leu Asp Ser Ser Trp Gly Leu
65                  70                  75                  80
Val Arg Asn Thr Ala Arg Phe Ile Glu Asp Tyr Gly Trp Tyr Lys Gly
                85                  90                  95
Glu Phe Phe Arg Glu Leu Val Ala Gly Tyr Ile Lys Glu Lys Thr Gly
            100                 105                 110
Asn Ser Glu Ser Thr Phe Lys Asp Leu Ala Lys Ser Lys Asp Phe Arg
        115                 120                 125
Gly Leu Ser Leu Ile Gly Ser Asp Leu Ser Thr Gly Tyr Ser Lys Val
    130                 135                 140
Phe Ser Asn Glu Phe Thr Pro Asn Val Lys Val Ala Asp Ala Ala Arg
145                 150                 155                 160
Ile Ser Met Ser Ile Pro Leu Phe Phe Lys Ala Val Arg Gly Val Asn
                165                 170                 175
Gly Asp Gly His Ile Tyr Val Asp Gly Gly Leu Leu Asp Asn Tyr Ala
            180                 185                 190
Ile Lys Val Phe Asp Arg Val Asn Tyr Val Lys Asn Lys Asn Asn Val
        195                 200                 205
Arg Tyr Thr Glu Tyr Tyr Glu Lys Thr Asn Lys Ser Leu Lys Ser Lys
    210                 215                 220
Asn Lys Leu Thr Asn Glu Tyr Val Tyr Asn Lys Glu Thr Leu Gly Phe
225                 230                 235                 240
Arg Leu Asp Ala Lys Glu Gln Ile Glu Met Phe Leu Asp His Ser Ile
                245                 250                 255
Glu Pro Lys Ala Lys Asp Ile Asp Ser Leu Phe Ser Tyr Thr Lys Ala
            260                 265                 270
Leu Val Thr Thr Leu Ile Asp Phe Gln Asn Asn Val His Leu His Ser
        275                 280                 285
Asp Asp Trp Gln Arg Thr Val Tyr Ile Asp Ser Leu Gly Ile Ser Ser
    290                 295                 300
Thr Asp Phe Gly Ile Ser Asp Ser Lys Lys Gln Lys Leu Val Asp Ser
305                 310                 315                 320
Gly Ile Leu His Thr Gln Lys Tyr Leu Asp Trp Tyr Asn Asn Asp Glu
                325                 330                 335
Glu Lys Ala Asn Lys
            340

<210> 79
<211> 1701
<212> DNA
```

<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 79
atgagaaatt tcagcaaggg attgaccagt attttgctta gcatagcgac atccaccagt 60
gcgatggcct ttacccagat cggggccggc ggagcgattc cgatgggcca tgagtggcta 120
acccgccgct cggcgctgga actgctgaat gccgacaatc tggtcggcaa tgacccggcc 180
gacccacgct tgggctggag cgaaggtctc gccaacaatc tcgatctctc gaatgcccag 240
aacgaagtgc agcgcatcaa gagcattacc aagagccacg ccctgtatga gccgcgttac 300
gatgacgttt tcgccgccat cgtcggcgag cgctggttg ataccgccgg tttcaacgtg 360
gccaaggcca ccgtcggcaa gatcgattgc ttcagcgccg tcgcgcaaga gcccgccgat 420
gtgcaacaag accatttcat gcgccgttat gacgacgtgg gtggacaagg gggcgtgaac 480
gctgcccgcc gcgcgcagca gcgctttatc aatcacttcg tcaacgcagc catggccgaa 540
gagaagagca tcaaggcatg ggatggcggc ggttattctt cgctggaaaa agtcagccac 600
aactacttct tgtttggccg cgccgttcat ttgttccagg attctttcag ccccgaacac 660
accgtgcgcc tgcctgaaga caattacgtc aaagtccgtc aggtcaaggc gtatctctgc 720
tctgaaggtg ccgaacagca tacgcacaac acgcaagatg ccatcaactt caccagcggc 780
gatgtcatct ggaaacagaa cacccgtctg gatgcaggct ggagcaccta caaggccagc 840
aacatgaagc cggtggcatt ggttgccctc gaagccagca aagatttgtg ggccgccttt 900
attcgcacca tggccgtttc ccgcgaggag cgtcgcgccg tcgccgaaca ggaagcgcag 960
gctctcgtca atcactggtt gtcgttcgac gaacaggaaa tgctgaactg gtacgaagaa 1020
gaagagcacc gcgatcatac gtacgtcaag gaacccggcc agagcggccc aggttcgtcg 1080
ttattcgatt gcatggttgg tctgggtgtg gcctcgggca gtcaggcgca acgggtggcg 1140
gaactcgatc agcaacgccg ccaatgtttg ttcaacgtca aggccgctac tggctatggc 1200
gatctgaatg atccacacat ggatattccg tacaactggc aatgggtgtc gtcgacgcaa 1260
tggaaaatcc ctgcggccga ctggaaaatc ccgcagctgc ccgccgattc agggaaatca 1320
gtcgtcatca agaattcgat caatggcgat ccgctggtgg cacctgccgg gctcaagcac 1380
aacaccgatg tttacggtgc accgggtgag gcgattgaat tcattttcgt cggtgatttc 1440
aaccatgagg cgtatttccg caccaaggac aacgcggatc tgttcctgag ttacagcgcg 1500
gtatcgggca agggcttgct gtacaacacg cccaaccagg ccggttatcg tgttcagcct 1560
tatggtgtgc tgtggacgat tgagaatacc tactggaatg atttcctctg gtacaacagc 1620
tcgaacgacc gcatctatgt cagcggcacc ggcgctgcca acaagtcaca ctcccagtgg 1680
attattgacg gcttgcagtg a 1701

<210> 80
<211> 566
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(23)

<400> 80
Met Arg Asn Phe Ser Lys Gly Leu Thr Ser Ile Leu Leu Ser Ile Ala
1 5 10 15
Thr Ser Thr Ser Ala Met Ala Phe Thr Gln Ile Gly Ala Gly Gly Ala
20 25 30
Ile Pro Met Gly His Glu Trp Leu Thr Arg Arg Ser Ala Leu Glu Leu
35 40 45
Leu Asn Ala Asp Asn Leu Val Gly Asn Asp Pro Ala Asp Pro Arg Leu
50 55 60
Gly Trp Ser Glu Gly Leu Ala Asn Asn Leu Asp Leu Ser Asn Ala Gln

65 70 75 80
Asn Glu Val Gln Arg Ile Lys Ser Ile Thr Lys Ser His Ala Leu Tyr
85 90 95
Glu Pro Arg Tyr Asp Asp Val Phe Ala Ala Ile Val Gly Glu Arg Trp
100 105 110
Val Asp Thr Ala Gly Phe Asn Val Ala Lys Ala Thr Val Gly Lys Ile
115 120 125
Asp Cys Phe Ser Ala Val Ala Gln Glu Pro Ala Asp Val Gln Gln Asp
130 135 140
His Phe Met Arg Arg Tyr Asp Asp Val Gly Gly Gln Gly Gly Val Asn
145 150 155 160
Ala Ala Arg Arg Ala Gln Gln Arg Phe Ile Asn His Phe Val Asn Ala
165 170 175
Ala Met Ala Glu Glu Lys Ser Ile Lys Ala Trp Asp Gly Gly Gly Tyr
180 185 190
Ser Ser Leu Glu Lys Val Ser His Asn Tyr Phe Leu Phe Gly Arg Ala
195 200 205
Val His Leu Phe Gln Asp Ser Phe Ser Pro Glu His Thr Val Arg Leu
210 215 220
Pro Glu Asp Asn Tyr Val Lys Val Arg Gln Val Lys Ala Tyr Leu Cys
225 230 235 240
Ser Glu Gly Ala Glu Gln His Thr His Asn Thr Gln Asp Ala Ile Asn
245 250 255
Phe Thr Ser Gly Asp Val Ile Trp Lys Gln Asn Thr Arg Leu Asp Ala
260 265 270
Gly Trp Ser Thr Tyr Lys Ala Ser Asn Met Lys Pro Val Ala Leu Val
275 280 285
Ala Leu Glu Ala Ser Lys Asp Leu Trp Ala Ala Phe Ile Arg Thr Met
290 295 300
Ala Val Ser Arg Glu Glu Arg Arg Ala Val Ala Glu Gln Glu Ala Gln
305 310 315 320
Ala Leu Val Asn His Trp Leu Ser Phe Asp Glu Gln Glu Met Leu Asn
325 330 335
Trp Tyr Glu Glu Glu Glu His Arg Asp His Thr Tyr Val Lys Glu Pro
340 345 350
Gly Gln Ser Gly Pro Gly Ser Ser Leu Phe Asp Cys Met Val Gly Leu
355 360 365
Gly Val Ala Ser Gly Ser Gln Ala Gln Arg Val Ala Glu Leu Asp Gln
370 375 380
Gln Arg Arg Gln Cys Leu Phe Asn Val Lys Ala Ala Thr Gly Tyr Gly
385 390 395 400
Asp Leu Asn Asp Pro His Met Asp Ile Pro Tyr Asn Trp Gln Trp Val
405 410 415
Ser Ser Thr Gln Trp Lys Ile Pro Ala Ala Asp Trp Lys Ile Pro Gln
420 425 430
Leu Pro Ala Asp Ser Gly Lys Ser Val Val Ile Lys Asn Ser Ile Asn
435 440 445
Gly Asp Pro Leu Val Ala Pro Ala Gly Leu Lys His Asn Thr Asp Val
450 455 460
Tyr Gly Ala Pro Gly Glu Ala Ile Glu Phe Ile Phe Val Gly Asp Phe
465 470 475 480
Asn His Glu Ala Tyr Phe Arg Thr Lys Asp Asn Ala Asp Leu Phe Leu
485 490 495
Ser Tyr Ser Ala Val Ser Gly Lys Gly Leu Leu Tyr Asn Thr Pro Asn
500 505 510
Gln Ala Gly Tyr Arg Val Gln Pro Tyr Gly Val Leu Trp Thr Ile Glu
515 520 525

Asn Thr Tyr Trp Asn Asp Phe Leu Trp Tyr Asn Ser Ser Asn Asp Arg
    530   535   540
Ile Tyr Val Ser Gly Thr Gly Ala Ala Asn Lys Ser His Ser Gln Trp
545   550   555   560
Ile Ile Asp Gly Leu Gln
                565

<210> 81
<211> 1422
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 81
atgaaaaaga aattatgtac aatggctctt gtaacagcaa tatcttctgg tgttgttacg 60
attccaacag aagcacaagc ttgtggaata ggcgaagtaa tgaaacagga gaaccaagag 120
cacaaacgtg tgaaaagatg gtctgcggag catccgcatc attcaaatga aagtacacat 180
ttatggattg cacgaaatgc gattcaaatt atgagtcgta atcaagataa gacggttcaa 240
gaaaatgaat tacaattttt aaatactcct gaatataagg agttatttga aagaggtctt 300
tatgatgctg attaccttga tgaatttaac gatggaggta caggtacaat cggcattgat 360
gggctaatta gaggagggtg gaaatctcat ttttacgatc ccgatacaag aaagaactat 420
aaaggggaag aagaaccaac agctctttca caaggagata aatattttaa attagcaggt 480
gaatacttta agaagggcga ccaaaaacaa gctttttatt atttaggtgt tgcaacgcat 540
tactttacag atgctactca accaatgcat gctgctaatt ttacagccgt cgacacgagt 600
gctttaaagt ttcatagcgc ttttgaaaat tatgtgacga caattcagac acagtatgaa 660
gtatctgatg gtgagggcgt atataattta gtgaattcta atgatccaaa acagtggatc 720
catgaaacag cgagactcgc aaaagtggaa atcgggaaca ttaccaatga cgagattaaa 780
tctcactata ataaaggaaa caatgctctt tggcaacaag aagttatgcc agctgtccag 840
aggagtttag agaacgcaca aagaaacacg gcgggattta ttcatttatg gtttaaaaca 900
tttgttggca atactgccgc tgaagaaatt gaaaatactg tagtgaaaga ttctaaagga 960
gaagcaatac aagataataa aaaatacttc gtagtgccaa gtgagtttct aaatagaggt 1020
ttgacctttg aagtatatgc aaggaatgac tatgcactat tatctaatta cgtagatgat 1080
agtaaagttc atggtacgcc agttcagttt gtatttgata aagataataa cggtatcctt 1140
catcgaggag aaagtgtact gctgaaaatg acgcaatcta actatgataa ttacgtattt 1200
ctaaactact ctaacttgac aaactgggta catcttgcgc aacaaaaaac aaatactgca 1260
cagtttaaag tgtatccaaa tccgaataac ccatctgaat attacctata tacagatgga 1320
tacccagtaa attatcaaga aaatggtaac ggaaagagct ggattgtgtt aggaaagaaa 1380
acagatacac caaaagcttg gaaatttata caggctgaat ag 1422

<210> 82
<211> 473
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(25)

<400> 82
Met Lys Lys Lys Leu Cys Thr Met Ala Leu Val Thr Ala Ile Ser Ser
 1   5   10   15
Gly Val Val Thr Ile Pro Thr Glu Ala Gln Ala Cys Gly Ile Gly Glu
            20   25   30

Val Met Lys Gln Glu Asn Gln Glu His Lys Arg Val Lys Arg Trp Ser
35 40 45
Ala Glu His Pro His His Ser Asn Glu Ser Thr His Leu Trp Ile Ala
50 55 60
Arg Asn Ala Ile Gln Ile Met Ser Arg Asn Gln Asp Lys Thr Val Gln
65 70 75 80
Glu Asn Glu Leu Gln Phe Leu Asn Thr Pro Glu Tyr Lys Glu Leu Phe
85 90 95
Glu Arg Gly Leu Tyr Asp Ala Asp Tyr Leu Asp Glu Phe Asn Asp Gly
100 105 110
Gly Thr Gly Thr Ile Gly Ile Asp Gly Leu Ile Arg Gly Gly Trp Lys
115 120 125
Ser His Phe Tyr Asp Pro Asp Thr Arg Lys Asn Tyr Lys Gly Glu Glu
130 135 140
Glu Pro Thr Ala Leu Ser Gln Gly Asp Lys Tyr Phe Lys Leu Ala Gly
145 150 155 160
Glu Tyr Phe Lys Lys Gly Asp Gln Lys Gln Ala Phe Tyr Tyr Leu Gly
165 170 175
Val Ala Thr His Tyr Phe Thr Asp Ala Thr Gln Pro Met His Ala Ala
180 185 190
Asn Phe Thr Ala Val Asp Thr Ser Ala Leu Lys Phe His Ser Ala Phe
195 200 205
Glu Asn Tyr Val Thr Thr Ile Gln Thr Gln Tyr Glu Val Ser Asp Gly
210 215 220
Glu Gly Val Tyr Asn Leu Val Asn Ser Asn Asp Pro Lys Gln Trp Ile
225 230 235 240
His Glu Thr Ala Arg Leu Ala Lys Val Glu Ile Gly Asn Ile Thr Asn
245 250 255
Asp Glu Ile Lys Ser His Tyr Asn Lys Gly Asn Asn Ala Leu Trp Gln
260 265 270
Gln Glu Val Met Pro Ala Val Gln Arg Ser Leu Glu Asn Ala Gln Arg
275 280 285
Asn Thr Ala Gly Phe Ile His Leu Trp Phe Lys Thr Phe Val Gly Asn
290 295 300
Thr Ala Ala Glu Glu Ile Glu Asn Thr Val Val Lys Asp Ser Lys Gly
305 310 315 320
Glu Ala Ile Gln Asp Asn Lys Lys Tyr Phe Val Val Pro Ser Glu Phe
325 330 335
Leu Asn Arg Gly Leu Thr Phe Glu Val Tyr Ala Arg Asn Asp Tyr Ala
340 345 350
Leu Leu Ser Asn Tyr Val Asp Asp Ser Lys Val His Gly Thr Pro Val
355 360 365
Gln Phe Val Phe Asp Lys Asp Asn Asn Gly Ile Leu His Arg Gly Glu
370 375 380
Ser Val Leu Leu Lys Met Thr Gln Ser Asn Tyr Asp Asn Tyr Val Phe
385 390 395 400
Leu Asn Tyr Ser Asn Leu Thr Asn Trp Val His Leu Ala Gln Gln Lys
405 410 415
Thr Asn Thr Ala Gln Phe Lys Val Tyr Pro Asn Pro Asn Asn Pro Ser
420 425 430
Glu Tyr Tyr Leu Tyr Thr Asp Gly Tyr Pro Val Asn Tyr Gln Glu Asn
435 440 445
Gly Asn Gly Lys Ser Trp Ile Val Leu Gly Lys Lys Thr Asp Thr Pro
450 455 460
Lys Ala Trp Lys Phe Ile Gln Ala Glu
465 470

<210> 83
<211> 1290
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 83
```
atgaaaaaga tagtgattta ttcatttgta gcaggggtta tgacatcagg cggcgtattt     60
gccgccagtg acaatattgt ggagacgtcg accccaccac agcatcaggc cccaagcaga    120
caggacaggg cattattcgc gggtgataca acaacctata taaaatgtgt ctacaaagtg    180
gatggccagg atgacagcaa tccatcctca tcttggttat gggcgaaagt gggtagcaac    240
tatgcgaagc tgaaggggta ttggtataat tcaatgccgc tggcaaacat gttttacact    300
gaagtaccct atgcagaggt gatggacttg tgtaatagca ccctgaaggc ggtaggtgcc    360
aactccactc ttgttattcc atatgcatcg gattacaccc tgtcctatta ctatgtgatt    420
tggaatcaag ggctaaccca gccggttatc aacgttggcg gcagagagct tgaccgtatg    480
gtggtctttg gtgacagctt gagcgatacc gtcaatgtct ataacggctc gtacggtacc    540
gtgccgaata gtacctcctg gttattgggc catttctcta acggaaagct ttggcatgaa    600
tacctttcca cggtattgaa tctgcctagc tatgtgtggg cgactggcaa tgcggagagt    660
ggagagaaac ccttctttaa cggattcagt aagcaggtgg attctttcag ggattatcac    720
gctcgcacta aaggctacga tattagcaag acgttgttta ccgttctgtt tggtggaaat    780
gattttataa cggggggaaa aagcgccgat gaggtcattg agcaatatac ggtgtcattg    840
aactacttgg ctcaactagg ggcgaagcag gttgcaattt tccgcttgcc agatttttca    900
gtgataccca gcgtttcaac gtggacagag gctgataagg acaaactgag agagaatagt    960
gttcagttta atgaccaagc cgagaagctg atcgctaaac taaacgcggc acatccccaa   1020
acgacgtttt atacgctgag gttggatgac gcttttaagc aggtgttgga aaacagcgac   1080
caatacggct ttgttaataa gactgatacc tgcctggata tttcccaagg cggatacaac   1140
tatgccattg ggcccgcgc gaaaacggca tgtaagagca gcaatgcggc gtttgtattc   1200
tgggacaata tgcatccgac caccaaaaca cacggattgt tggccgatct tttaaaagat   1260
gatgtggtac gcggcctcgc tgcgccatga                                    1290
```

<210> 84
<211> 429
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(22)

<400> 84
```
Met Lys Lys Ile Val Ile Tyr Ser Phe Val Ala Gly Val Met Thr Ser
 1               5                  10                  15
Gly Gly Val Phe Ala Ala Ser Asp Asn Ile Val Glu Thr Ser Thr Pro
            20                  25                  30
Pro Gln His Gln Ala Pro Ser Arg Gln Asp Arg Ala Leu Phe Ala Gly
        35                  40                  45
Asp Thr Thr Thr Tyr Ile Lys Cys Val Tyr Lys Val Asp Gly Gln Asp
    50                  55                  60
Asp Ser Asn Pro Ser Ser Ser Trp Leu Trp Ala Lys Val Gly Ser Asn
65                  70                  75                  80
Tyr Ala Lys Leu Lys Gly Tyr Trp Tyr Asn Ser Met Pro Leu Ala Asn
                85                  90                  95
Met Phe Tyr Thr Glu Val Pro Tyr Ala Glu Val Met Asp Leu Cys Asn
```

```
            100                 105                 110
Ser Thr Leu Lys Ala Val Gly Ala Asn Ser Thr Leu Val Ile Pro Tyr
        115                 120                 125
Ala Ser Asp Tyr Thr Leu Ser Tyr Tyr Tyr Val Ile Trp Asn Gln Gly
    130                 135                 140
Ala Asn Gln Pro Val Ile Asn Val Gly Gly Arg Glu Leu Asp Arg Met
145                 150                 155                 160
Val Val Phe Gly Asp Ser Leu Ser Asp Thr Val Asn Val Tyr Asn Gly
                165                 170                 175
Ser Tyr Gly Thr Val Pro Asn Ser Thr Ser Trp Leu Leu Gly His Phe
            180                 185                 190
Ser Asn Gly Lys Leu Trp His Glu Tyr Leu Ser Thr Val Leu Asn Leu
        195                 200                 205
Pro Ser Tyr Val Trp Ala Thr Gly Asn Ala Glu Ser Gly Glu Lys Pro
    210                 215                 220
Phe Phe Asn Gly Phe Ser Lys Gln Val Asp Ser Phe Arg Asp Tyr His
225                 230                 235                 240
Ala Arg Thr Lys Gly Tyr Asp Ile Ser Lys Thr Leu Phe Thr Val Leu
                245                 250                 255
Phe Gly Gly Asn Asp Phe Ile Thr Gly Gly Lys Ser Ala Asp Glu Val
            260                 265                 270
Ile Glu Gln Tyr Thr Val Ser Leu Asn Tyr Leu Ala Gln Leu Gly Ala
        275                 280                 285
Lys Gln Val Ala Ile Phe Arg Leu Pro Asp Phe Ser Val Ile Pro Ser
    290                 295                 300
Val Ser Thr Trp Thr Glu Ala Asp Lys Asp Lys Leu Arg Glu Asn Ser
305                 310                 315                 320
Val Gln Phe Asn Asp Gln Ala Glu Lys Leu Ile Ala Lys Leu Asn Ala
                325                 330                 335
Ala His Pro Gln Thr Thr Phe Tyr Thr Leu Arg Leu Asp Asp Ala Phe
            340                 345                 350
Lys Gln Val Leu Glu Asn Ser Asp Gln Tyr Gly Phe Val Asn Lys Thr
        355                 360                 365
Asp Thr Cys Leu Asp Ile Ser Gln Gly Gly Tyr Asn Tyr Ala Ile Gly
    370                 375                 380
Ala Arg Ala Lys Thr Ala Cys Lys Ser Ser Asn Ala Ala Phe Val Phe
385                 390                 395                 400
Trp Asp Asn Met His Pro Thr Thr Lys Thr His Gly Leu Leu Ala Asp
                405                 410                 415
Leu Leu Lys Asp Asp Val Val Arg Gly Leu Ala Ala Pro
            420                 425
```

<210> 85
<211> 1038
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 85

```
atgacaacac aatttagaaa cttgatattt gaaggcggcg gtgtaaaagg tgttgcttac      60
attggcgcca tgcagattct tgaaaatcgt ggcgtgttgc aagatattcg ccgagtcgga     120
gggtgcagtg cgggtgcgat taacgcgctg atttttgcgc taggttacac ggtccgtgaa     180
caaaaagaga tcttacaagc caccgatttt aaccagttta tggataactc ttggggggtt     240
attcgtgata ttcgcaggct tgctcgagac tttggctgga ataagggtga tttctttagt     300
agctggatag gtgatttgat tcatcgtcgt ttggggaatc gccgagcgac gttcaaagat     360
```

```
ctgcaaaagg ccaagcttcc tgatctttat gtcatcggta ctaatctgtc tacagggttt    420
gcagaggtgt tttctgccga aagacacccc gatatggagc tggcgacagc ggtgcgtatc    480
tccatgtcga taccgctgtt ctttgcggcc gtgcgtcacg gtgatcgaca agatgtgtat    540
gtcgatgggg gtgttcaact taactatccg attaaactgt ttgatcggga gcgttacatt    600
gatttggcca aagatcccgg tgccgttcgg cgaacgggtt attacaacaa agaaaacgct    660
cgctttcagc ttgatcggcc gggccatagc ccctatgttt acaatcgcca gaccttgggt    720
ttgcgactgg atagtcgcga ggagataggg ctctttcgtt atgacgaacc cctcaagggc    780
aaacccatta agtccttcac tgactacgct cgacaacttt tcggtgcgtt gatgaatgca    840
caggaaaaga ttcatctaca tggcgatgat tggcaacgca cgatctatat cgatacattg    900
gatgtgggta cgacggactt caatctttct gatgcaacta agcaagcact gattgagcaa    960
ggaattaacg gcaccgaaaa ttatttcgag tggtttgata atccgttaga gaagcctgtg   1020
aatagagtgg agtcatag                                                 1038
```

<210> 86
<211> 345
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 86

```
Met Thr Thr Gln Phe Arg Asn Leu Ile Phe Glu Gly Gly Gly Val Lys
1               5                   10                  15
Gly Val Ala Tyr Ile Gly Ala Met Gln Ile Leu Glu Asn Arg Gly Val
            20                  25                  30
Leu Gln Asp Ile Arg Arg Val Gly Gly Cys Ser Ala Gly Ala Ile Asn
        35                  40                  45
Ala Leu Ile Phe Ala Leu Gly Tyr Thr Val Arg Glu Gln Lys Glu Ile
    50                  55                  60
Leu Gln Ala Thr Asp Phe Asn Gln Phe Met Asp Asn Ser Trp Gly Val
65                  70                  75                  80
Ile Arg Asp Ile Arg Arg Leu Ala Arg Asp Phe Gly Trp Asn Lys Gly
                85                  90                  95
Asp Phe Phe Ser Ser Trp Ile Gly Asp Leu Ile His Arg Arg Leu Gly
            100                 105                 110
Asn Arg Arg Ala Thr Phe Lys Asp Leu Gln Lys Ala Lys Leu Pro Asp
        115                 120                 125
Leu Tyr Val Ile Gly Thr Asn Leu Ser Thr Gly Phe Ala Glu Val Phe
    130                 135                 140
Ser Ala Glu Arg His Pro Asp Met Glu Leu Ala Thr Ala Val Arg Ile
145                 150                 155                 160
Ser Met Ser Ile Pro Leu Phe Phe Ala Ala Val Arg His Gly Asp Arg
                165                 170                 175
Gln Asp Val Tyr Val Asp Gly Gly Val Gln Leu Asn Tyr Pro Ile Lys
            180                 185                 190
Leu Phe Asp Arg Glu Arg Tyr Ile Asp Leu Ala Lys Asp Pro Gly Ala
        195                 200                 205
Val Arg Arg Thr Gly Tyr Tyr Asn Lys Glu Asn Ala Arg Phe Gln Leu
    210                 215                 220
Asp Arg Pro Gly His Ser Pro Tyr Val Tyr Asn Arg Gln Thr Leu Gly
225                 230                 235                 240
Leu Arg Leu Asp Ser Arg Glu Glu Ile Gly Leu Phe Arg Tyr Asp Glu
                245                 250                 255
Pro Leu Lys Gly Lys Pro Ile Lys Ser Phe Thr Asp Tyr Ala Arg Gln
            260                 265                 270
Leu Phe Gly Ala Leu Met Asn Ala Gln Glu Lys Ile His Leu His Gly
```

275 280 285
Asp Asp Trp Gln Arg Thr Ile Tyr Ile Asp Thr Leu Asp Val Gly Thr
290 295 300
Thr Asp Phe Asn Leu Ser Asp Ala Thr Lys Gln Ala Leu Ile Glu Gln
305 310 315 320
Gly Ile Asn Gly Thr Glu Asn Tyr Phe Glu Trp Phe Asp Asn Pro Leu
325 330 335
Glu Lys Pro Val Asn Arg Val Glu Ser
340 345

<210> 87
<211> 870
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 87
atgtcaaaga aactcgtaat atcggtagcg ggcggcggag cactcggaat cggaccactc 60
gcattcctgt gcaagattga acagatgctg ggaaagaaga taccccaggt tgcgcaggca 120
tacgccggca cttcaaccgg agcaataatt gcggcaggac tggccgaagg ctactccgcg 180
catgaactgt tcgacctata caaatcaaat ctcagcaaga tattcaccaa atacagctgg 240
tacaaacgcc tgcagccaac gtgtcctaca tatgacaaca gtaacctaaa gaaattactg 300
aaggacaaat tcaagggcaa ggtcggcgac tggaaaactc ccgtatacat cccggcaaca 360
cacatgaacg gccaatccgt agaaaaggtg tgggacttgg gtgacaagaa tgttgacaag 420
tggtttgcca ttctgacaag taccgcggca ccaacctatt tcgactgcat atacgacgac 480
gagaagaact gctacatcga tggtggcatg tggtgcaacg caccaatcga tgtgcttaat 540
gcaggcctga tcaagtccgg ctggtccaac tacaaggtcc tggacctgga gaccggcatg 600
gacacaccga atacggaaag cggaaacaag acacttctcg gatgggggga atacatcata 660
agcaactggg tagcccgttc cagcaagtcc ggcgaatacg aggtaaaggc cataatcggg 720
gaagacaatg tatgtgttgc ccgtccatac gtaagcaaga aaccgaagat ggatgacgtg 780
gacagcaaga cgctggatga agtcgtggat atctgggaaa actacttcta cgccaagcag 840
aaagacatcg catcgtggct gaaaatctag 870

<210> 88
<211> 289
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 88
Met Ser Lys Lys Leu Val Ile Ser Val Ala Gly Gly Gly Ala Leu Gly
1 5 10 15
Ile Gly Pro Leu Ala Phe Leu Cys Lys Ile Glu Gln Met Leu Gly Lys
20 25 30
Lys Ile Pro Gln Val Ala Gln Ala Tyr Ala Gly Thr Ser Thr Gly Ala
35 40 45
Ile Ile Ala Ala Gly Leu Ala Glu Gly Tyr Ser Ala His Glu Leu Phe
50 55 60
Asp Leu Tyr Lys Ser Asn Leu Ser Lys Ile Phe Thr Lys Tyr Ser Trp
65 70 75 80
Tyr Lys Arg Leu Gln Pro Thr Cys Pro Thr Tyr Asp Asn Ser Asn Leu
85 90 95
Lys Lys Leu Leu Lys Asp Lys Phe Lys Gly Lys Val Gly Asp Trp Lys

```
            100                 105                 110
Thr Pro Val Tyr Ile Pro Ala Thr His Met Asn Gly Gln Ser Val Glu
        115                 120                 125
Lys Val Trp Asp Leu Gly Asp Lys Asn Val Asp Lys Trp Phe Ala Ile
    130                 135                 140
Leu Thr Ser Thr Ala Ala Pro Thr Tyr Phe Asp Cys Ile Tyr Asp Asp
145                 150                 155                 160
Glu Lys Asn Cys Tyr Ile Asp Gly Gly Met Trp Cys Asn Ala Pro Ile
                165                 170                 175
Asp Val Leu Asn Ala Gly Leu Ile Lys Ser Gly Trp Ser Asn Tyr Lys
            180                 185                 190
Val Leu Asp Leu Glu Thr Gly Met Asp Thr Pro Asn Thr Glu Ser Gly
        195                 200                 205
Asn Lys Thr Leu Leu Gly Trp Gly Glu Tyr Ile Ile Ser Asn Trp Val
    210                 215                 220
Ala Arg Ser Ser Lys Ser Gly Glu Tyr Glu Val Lys Ala Ile Ile Gly
225                 230                 235                 240
Glu Asp Asn Val Cys Val Ala Arg Pro Tyr Val Ser Lys Lys Pro Lys
                245                 250                 255
Met Asp Asp Val Asp Ser Lys Thr Leu Asp Glu Val Val Asp Ile Trp
            260                 265                 270
Glu Asn Tyr Phe Tyr Ala Lys Gln Lys Asp Ile Ala Ser Trp Leu Lys
        275                 280                 285
Ile
```

<210> 89
<211> 1422
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 89

```
atgaaaaaga aattatgtac actggctttt gtaacagcaa tatcttctat cgctatcaca      60
attccaacag aagcacaagc ttgtggaata ggcgaagtaa tgaaacagga gaaccaagag     120
cacaaacgtg tgaagagatg gtctgcggaa catccacatc atcctaatga aagtacgcac     180
ttatggattg cgcgaaatgc aattcaaata atggcccgta atcaagataa gacggttcaa     240
gaaaatgaat tacaattttt aaatactcct gaatataagg agttatttga aagaggtctt     300
tatgatgctg attaccttga tgaatttaac gatggaggta caggtacaat cggcattgat     360
gggctaatta aaggagggtg gaaatctcat ttttacgatc ccgatacgag aaagaactat     420
aaaggggaag aagaaccaac agctctctct caaggagata aatattttaa attagcaggc     480
gattacttta agaaagagga ttggaaacaa gctttctatt atttaggtgt tgcgacgcac     540
tacttcacag atgctactca gccaatgcat gctgctaatt ttacagccgt cgacacgagt     600
gctttaaagt ttcatagcgc ttttgaaaat tatgtgacga caattcagac acagtatgaa     660
gtatctgatg gtgagggcgt atataattta gtgaattcta atgatccaaa acagtggatc     720
catgaaacag cgagactcgc aaaagtggaa atcgggaaca ttaccaatga cgagattaaa     780
tctcactata ataaaggaaa caatgctctt tggcaacaag aagttatgcc agctgtccag     840
aggagtttag agaacgcaca aagaaacacg gcgggattta ttcatttatg gtttaaaaca     900
tttgttggca atactgccgc tgaagaaatt gaaaatactg tagtgaaaga ttctaaagga     960
gaagcaatac aagataataa aaaatacttc gtagtgccaa gtgagtttct aaatagaggt    1020
ttgacctttg aagtatatgc aaggaatgac tatgcactat tatctaatta cgtagatgat    1080
agtaaagttc atggtacgcc agttcagttt gtatttgata aagataataa cggtatcctt    1140
catcgaggag aaagtatact gctgaaaatg acgcaatcta actatgataa ttacgtattt    1200
ctaaactact ctaacttgac aaactgggta catcttgcgc aacaaaaaac aaatactgca    1260
cagtttaaag tgtatccaaa tccgaataac ccatctgaat attacctata tacagatgga    1320
```

tacccagtaa attatcaaga aaatggtaac ggaaagagct ggattgtgtt aggaaagaaa 1380
acagatacac caaaagcttg gaaatttata caggctgaat ag 1422

<210> 90
<211> 473
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(25)

<400> 90
Met Lys Lys Lys Leu Cys Thr Leu Ala Phe Val Thr Ala Ile Ser Ser
1 5 10 15
Ile Ala Ile Thr Ile Pro Thr Glu Ala Gln Ala Cys Gly Ile Gly Glu
20 25 30
Val Met Lys Gln Glu Asn Gln Glu His Lys Arg Val Lys Arg Trp Ser
35 40 45
Ala Glu His Pro His His Pro Asn Glu Ser Thr His Leu Trp Ile Ala
50 55 60
Arg Asn Ala Ile Gln Ile Met Ala Arg Asn Gln Asp Lys Thr Val Gln
65 70 75 80
Glu Asn Glu Leu Gln Phe Leu Asn Thr Pro Glu Tyr Lys Glu Leu Phe
85 90 95
Glu Arg Gly Leu Tyr Asp Ala Asp Tyr Leu Asp Glu Phe Asn Asp Gly
100 105 110
Gly Thr Gly Thr Ile Gly Ile Asp Gly Leu Ile Lys Gly Gly Trp Lys
115 120 125
Ser His Phe Tyr Asp Pro Asp Thr Arg Lys Asn Tyr Lys Gly Glu Glu
130 135 140
Glu Pro Thr Ala Leu Ser Gln Gly Asp Lys Tyr Phe Lys Leu Ala Gly
145 150 155 160
Asp Tyr Phe Lys Lys Glu Asp Trp Lys Gln Ala Phe Tyr Tyr Leu Gly
165 170 175
Val Ala Thr His Tyr Phe Thr Asp Ala Thr Gln Pro Met His Ala Ala
180 185 190
Asn Phe Thr Ala Val Asp Thr Ser Ala Leu Lys Phe His Ser Ala Phe
195 200 205
Glu Asn Tyr Val Thr Thr Ile Gln Thr Gln Tyr Glu Val Ser Asp Gly
210 215 220
Glu Gly Val Tyr Asn Leu Val Asn Ser Asn Asp Pro Lys Gln Trp Ile
225 230 235 240
His Glu Thr Ala Arg Leu Ala Lys Val Glu Ile Gly Asn Ile Thr Asn
245 250 255
Asp Glu Ile Lys Ser His Tyr Asn Lys Gly Asn Asn Ala Leu Trp Gln
260 265 270
Gln Glu Val Met Pro Ala Val Gln Arg Ser Leu Glu Asn Ala Gln Arg
275 280 285
Asn Thr Ala Gly Phe Ile His Leu Trp Phe Lys Thr Phe Val Gly Asn
290 295 300
Thr Ala Ala Glu Glu Ile Glu Asn Thr Val Val Lys Asp Ser Lys Gly
305 310 315 320
Glu Ala Ile Gln Asp Asn Lys Lys Tyr Phe Val Val Pro Ser Glu Phe
325 330 335

Leu Asn Arg Gly Leu Thr Phe Glu Val Tyr Ala Arg Asn Asp Tyr Ala
340 345 350
Leu Leu Ser Asn Tyr Val Asp Asp Ser Lys Val His Gly Thr Pro Val
355 360 365
Gln Phe Val Phe Asp Lys Asp Asn Asn Gly Ile Leu His Arg Gly Glu
370 375 380
Ser Ile Leu Leu Lys Met Thr Gln Ser Asn Tyr Asp Asn Tyr Val Phe
385 390 395 400
Leu Asn Tyr Ser Asn Leu Thr Asn Trp Val His Leu Ala Gln Gln Lys
405 410 415
Thr Asn Thr Ala Gln Phe Lys Val Tyr Pro Asn Pro Asn Asn Pro Ser
420 425 430
Glu Tyr Tyr Leu Tyr Thr Asp Gly Tyr Pro Val Asn Tyr Gln Glu Asn
435 440 445
Gly Asn Gly Lys Ser Trp Ile Val Leu Gly Lys Lys Thr Asp Thr Pro
450 455 460
Lys Ala Trp Lys Phe Ile Gln Ala Glu
465 470

<210> 91
<211> 1035
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 91
atgacaaccc aatttagaaa cctgatcttt gagggcggcg gtgtaaaggg cattgcttac 60
gtcggagcaa tgcagattct tgaaaatcgt ggtgtattac aagatattca ccgagtcgga 120
ggttgtagtg cgggtgcgat taacgcgctg atttttgcgc tgggttacac agtccgtgag 180
caaaaagaga tcttacaaat taccgatttt aaccagttta tggataactc gtggggtgtt 240
attcgggata ttcgcaggct tgcgagagaa tttggctgga ataagggtaa cttctttaat 300
acctggatag gtgatttgat tcatcgtcgt ttgggtaatc gccgagccac gttcaaagat 360
ctgcaaaagg caaagcttcc tgatctttat gtcatcggta ctaatctgtc tacagggttt 420
gcagaggttt tttctgccga aagacacccc gatatggagc tggcgacagc ggtgcgtatc 480
tccatgtcga taccgctgtt ctttgcggcc gtgcgtcacg gtgatcgaca agatgtgtat 540
gtcgatgggg gtgtgcagct taactacccg atcaagctgt ttgatcgaac tcgttatatt 600
gacctcgcca aagatccggg tgctgctcgc cacacgggtt attacaataa agagaatgct 660
cgttttcagc ttgagcgacc gggccacagt ccttatgtgt acaatcgcca aacattaggc 720
ttgcgtcttg acagtcgtga agagatagcg ctgtttcgtt acgacgaacc tcttcagggt 780
aaacccatta agtccttcac tgactacgct cgacaacttt ttggtgcgct gaagaatgca 840
caggaaaaca ttcacctaca tggcgatgat tggcagcgca cggtctatat cgatacattg 900
gatgtgggta cgacggattt caatctttct gatgcaacca agcaagcact gattgaacag 960
ggaattaacg gcaccgaaaa ttatttcgag tggtttgata atccgtttga gaagcctgtg 1020
aatagagtgg agtaa 1035

<210> 92
<211> 344
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 92
Met Thr Thr Gln Phe Arg Asn Leu Ile Phe Glu Gly Gly Gly Val Lys

```
1               5                   10                  15
Gly Ile Ala Tyr Val Gly Ala Met Gln Ile Leu Glu Asn Arg Gly Val
            20                  25                  30
Leu Gln Asp Ile His Arg Val Gly Gly Cys Ser Ala Gly Ala Ile Asn
        35                  40                  45
Ala Leu Ile Phe Ala Leu Gly Tyr Thr Val Arg Glu Gln Lys Glu Ile
    50                  55                  60
Leu Gln Ile Thr Asp Phe Asn Gln Phe Met Asp Asn Ser Trp Gly Val
65                  70                  75                  80
Ile Arg Asp Ile Arg Arg Leu Ala Arg Glu Phe Gly Trp Asn Lys Gly
                85                  90                  95
Asn Phe Phe Asn Thr Trp Ile Gly Asp Leu Ile His Arg Arg Leu Gly
            100                 105                 110
Asn Arg Arg Ala Thr Phe Lys Asp Leu Gln Lys Ala Lys Leu Pro Asp
        115                 120                 125
Leu Tyr Val Ile Gly Thr Asn Leu Ser Thr Gly Phe Ala Glu Val Phe
    130                 135                 140
Ser Ala Glu Arg His Pro Asp Met Glu Leu Ala Thr Ala Val Arg Ile
145                 150                 155                 160
Ser Met Ser Ile Pro Leu Phe Phe Ala Ala Val Arg His Gly Asp Arg
                165                 170                 175
Gln Asp Val Tyr Val Asp Gly Gly Val Gln Leu Asn Tyr Pro Ile Lys
            180                 185                 190
Leu Phe Asp Arg Thr Arg Tyr Ile Asp Leu Ala Lys Asp Pro Gly Ala
        195                 200                 205
Ala Arg His Thr Gly Tyr Tyr Asn Lys Glu Asn Ala Arg Phe Gln Leu
    210                 215                 220
Glu Arg Pro Gly His Ser Pro Tyr Val Tyr Asn Arg Gln Thr Leu Gly
225                 230                 235                 240
Leu Arg Leu Asp Ser Arg Glu Glu Ile Ala Leu Phe Arg Tyr Asp Glu
                245                 250                 255
Pro Leu Gln Gly Lys Pro Ile Lys Ser Phe Thr Asp Tyr Ala Arg Gln
            260                 265                 270
Leu Phe Gly Ala Leu Lys Asn Ala Gln Glu Asn Ile His Leu His Gly
        275                 280                 285
Asp Asp Trp Gln Arg Thr Val Tyr Ile Asp Thr Leu Asp Val Gly Thr
    290                 295                 300
Thr Asp Phe Asn Leu Ser Asp Ala Thr Lys Gln Ala Leu Ile Glu Gln
305                 310                 315                 320
Gly Ile Asn Gly Thr Glu Asn Tyr Phe Glu Trp Phe Asp Asn Pro Phe
                325                 330                 335
Glu Lys Pro Val Asn Arg Val Glu
            340
```

<210> 93
<211> 963
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 93

```
gtgattactt tgataaaaaa atgtttatta gtattgacga tgactctatt atcaggggtt      60
ttcgtaccgc tgcagccatc atatgctact gaaaattatc caaatgattt taaactgttg     120
caacataatg tatttttatt gcctgaatca gtttcttatt ggggtcagga cgaacgtgca     180
gattatatga gtaatgcaga ttactttaag ggacatgatg ctctgctctt aaatgagctt     240
```

```
tttgacaatg gaaattcgaa cgtgctgcta atgaacttat ccaaggaata tacatatcaa      300
acgccagtgc ttggccgttc gatgagtgga tgggatgaaa ctagaggaag ctattctaat      360
tttgtacccg aagatggtgg tgtagcaatt atcagtaaat ggccaatcgt ggagaaaata      420
cagcatgttt acgcgaatgg ttgcggtgca gactattatg caaataaagg atttgtttat      480
gcaaaagtac aaaaagggga taaattctat catcttatca gcactcatgc tcaagccgaa      540
gataccgggt gtgatcaggg tgaaggagca gaaattcgtc attcacagtt tcaagaaatc      600
aacgacttta ttaaaaataa aaacattccg aaagatgaag tggtatttat tggtggtgac      660
tttaatgtga tgaagagtga cacaacagag tacaatagca tgttatcaac attaaatgtc      720
aatgcgccta ccgaatattt agggcataac tctacttggg acccagaaac gaacagcatt      780
acaggttaca attaccctga ttatgcgcca cagcatttag attatatttt tgtggaaaaa      840
gatcataaac aaccaagttc atgggtaaat gaaacgatta ctccgaagtc tccaacttgg      900
aaggcaatct atgagtataa tgattattcc gatcactatc ctgttaaagc atacgtaaaa      960
taa                                                                    963
```

```
<210> 94
<211> 320
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(29)

<400> 94
Met Ile Thr Leu Ile Lys Lys Cys Leu Leu Val Leu Thr Met Thr Leu
 1               5                  10                  15
Leu Ser Gly Val Phe Val Pro Leu Gln Pro Ser Tyr Ala Thr Glu Asn
            20                  25                  30
Tyr Pro Asn Asp Phe Lys Leu Leu Gln His Asn Val Phe Leu Leu Pro
        35                  40                  45
Glu Ser Val Ser Tyr Trp Gly Gln Asp Glu Arg Ala Asp Tyr Met Ser
    50                  55                  60
Asn Ala Asp Tyr Phe Lys Gly His Asp Ala Leu Leu Leu Asn Glu Leu
65                  70                  75                  80
Phe Asp Asn Gly Asn Ser Asn Val Leu Leu Met Asn Leu Ser Lys Glu
                85                  90                  95
Tyr Thr Tyr Gln Thr Pro Val Leu Gly Arg Ser Met Ser Gly Trp Asp
            100                 105                 110
Glu Thr Arg Gly Ser Tyr Ser Asn Phe Val Pro Glu Asp Gly Gly Val
        115                 120                 125
Ala Ile Ile Ser Lys Trp Pro Ile Val Glu Lys Ile Gln His Val Tyr
    130                 135                 140
Ala Asn Gly Cys Gly Ala Asp Tyr Tyr Ala Asn Lys Gly Phe Val Tyr
145                 150                 155                 160
Ala Lys Val Gln Lys Gly Asp Lys Phe Tyr His Leu Ile Ser Thr His
                165                 170                 175
Ala Gln Ala Glu Asp Thr Gly Cys Asp Gln Gly Glu Gly Ala Glu Ile
            180                 185                 190
Arg His Ser Gln Phe Gln Glu Ile Asn Asp Phe Ile Lys Asn Lys Asn
        195                 200                 205
Ile Pro Lys Asp Glu Val Val Phe Ile Gly Gly Asp Phe Asn Val Met
    210                 215                 220
Lys Ser Asp Thr Thr Glu Tyr Asn Ser Met Leu Ser Thr Leu Asn Val
225                 230                 235                 240
Asn Ala Pro Thr Glu Tyr Leu Gly His Asn Ser Thr Trp Asp Pro Glu
```

```
                245                     250                     255
Thr Asn Ser Ile Thr Gly Tyr Asn Tyr Pro Asp Tyr Ala Pro Gln His
            260                     265                     270
Leu Asp Tyr Ile Phe Val Glu Lys Asp His Lys Gln Pro Ser Ser Trp
        275                     280                     285
Val Asn Glu Thr Ile Thr Pro Lys Ser Pro Thr Trp Lys Ala Ile Tyr
    290                     295                     300
Glu Tyr Asn Asp Tyr Ser Asp His Tyr Pro Val Lys Ala Tyr Val Lys
305                     310                     315                     320
```

<210> 95
<211> 1038
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 95

```
atggcttcac aattcaggaa tctggtattt gaaggaggtg gtgtaaaagg gattgcgtac      60
ataggtgcga tgcaggtgct ggatcagcgc ggttatttgg gtgataacat caaacgcgtt     120
ggtggaacca gtgcaggtgc cataaatgcg ctgatttatt cgttaggata tgacatccac     180
gaacaacaag agatactgaa ctctacagat tttaaaaagt ttatggataa ctcttttgga     240
tttgtgaggg atttcagaag gctatggaat gaatttggat ggaatagagg agactttttt     300
cttaaatggt caggtgagct gatcaaaaat aaattgggca cctcaaaagc cacctttcag     360
gatttgaagg atgccggtca gccagatttg tatgtaattg gaacaaattt atcgacgggg     420
ttttccgaga ctttttcata tgaacgtcac cccgatatga ctcttgcaga agccgtaaga     480
atcagtatgt cgcttccgct gtttttcagg gctgtgcggt tgggcgacag gaatgatgta     540
tatgtggatg gtggggttca gctcaattac ccggtaaaac tatttgatcg tgaaaaatat     600
attgatatgg ataatgaggc ggctgcagca cgatttactg attattacaa caaagaaaat     660
gccagatttt cgctccagcg gcctggacga agcccctatg tatataatcg tcaaaccctt     720
ggtttgagac tggatacagc cgaagaaatt gcgcttttca ggtacgatga acccattcag     780
gggaaagaga tcaaacggtt tccggaatat gcaaaggctc tgatcggcgc actaatgcag     840
gtgcaggaaa acatacatct ccacagtgac gactggcagc gtacgctgta tatcaatacc     900
ctggatgtaa aaaccacaga ttttgaatta accgatgaga aaaaaaagga actggtagaa     960
cagggaatcc ttggcgcgga aacctatttc aaatggtttg aagacaggga tgaagtagtt    1020
gtaaaccgcc ttgcttag                                                 1038
```

<210> 96
<211> 345
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 96

```
Met Ala Ser Gln Phe Arg Asn Leu Val Phe Glu Gly Gly Gly Val Lys
1               5                   10                  15
Gly Ile Ala Tyr Ile Gly Ala Met Gln Val Leu Asp Gln Arg Gly Tyr
            20                  25                  30
Leu Gly Asp Asn Ile Lys Arg Val Gly Gly Thr Ser Ala Gly Ala Ile
        35                  40                  45
Asn Ala Leu Ile Tyr Ser Leu Gly Tyr Asp Ile His Glu Gln Gln Glu
    50                  55                  60
Ile Leu Asn Ser Thr Asp Phe Lys Lys Phe Met Asp Asn Ser Phe Gly
65                  70                  75                  80
```

```
Phe Val Arg Asp Phe Arg Arg Leu Trp Asn Glu Phe Gly Trp Asn Arg
                85                  90                  95
Gly Asp Phe Phe Leu Lys Trp Ser Gly Glu Leu Ile Lys Asn Lys Leu
            100                 105                 110
Gly Thr Ser Lys Ala Thr Phe Gln Asp Leu Lys Asp Ala Gly Gln Pro
        115                 120                 125
Asp Leu Tyr Val Ile Gly Thr Asn Leu Ser Thr Gly Phe Ser Glu Thr
    130                 135                 140
Phe Ser Tyr Glu Arg His Pro Asp Met Thr Leu Ala Glu Ala Val Arg
145                 150                 155                 160
Ile Ser Met Ser Leu Pro Leu Phe Phe Arg Ala Val Arg Leu Gly Asp
                165                 170                 175
Arg Asn Asp Val Tyr Val Asp Gly Gly Val Gln Leu Asn Tyr Pro Val
            180                 185                 190
Lys Leu Phe Asp Arg Glu Lys Tyr Ile Asp Met Asp Asn Glu Ala Ala
        195                 200                 205
Ala Ala Arg Phe Thr Asp Tyr Tyr Asn Lys Glu Asn Ala Arg Phe Ser
    210                 215                 220
Leu Gln Arg Pro Gly Arg Ser Pro Tyr Val Tyr Asn Arg Gln Thr Leu
225                 230                 235                 240
Gly Leu Arg Leu Asp Thr Ala Glu Glu Ile Ala Leu Phe Arg Tyr Asp
                245                 250                 255
Glu Pro Ile Gln Gly Lys Glu Ile Lys Arg Phe Pro Glu Tyr Ala Lys
            260                 265                 270
Ala Leu Ile Gly Ala Leu Met Gln Val Gln Glu Asn Ile His Leu His
        275                 280                 285
Ser Asp Asp Trp Gln Arg Thr Leu Tyr Ile Asn Thr Leu Asp Val Lys
    290                 295                 300
Thr Thr Asp Phe Glu Leu Thr Asp Glu Lys Lys Lys Glu Leu Val Glu
305                 310                 315                 320
Gln Gly Ile Leu Gly Ala Glu Thr Tyr Phe Lys Trp Phe Glu Asp Arg
                325                 330                 335
Asp Glu Val Val Val Asn Arg Leu Ala
            340                 345
```

<210> 97
<211> 1422
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 97

```
atgaaaagga aactatgtac atgggctctc gtaacagcaa tagcttctag tactgcggta      60
attccaacag cagcagaagc ttgtggatta ggagaagtaa tcaaacaaga gaatcaagag     120
cacaaacgtg tgaaaagatg gtctgcggag catccgcatc attcacatga aagtacccat     180
ttatggattg cacaaaatgc gattcaaatt atgagccgta atcaagataa gacggttcaa     240
gaaaatgaat tacaattttt aaatacccct gaatataagg agttatttga aagaggtctt     300
tatgatgctg attaccttga tgaatttaac gatggaggta caggtataat cggcattgat     360
gggctaattc gaggagggtg gaaatctcat ttctacgatc ccgatacaag aaagaactat     420
aaaggggagg aagaaccaac agctctttct caaggagata aatattttaa attagcaggt     480
gaatacttta agaagaatga ttggaaacag gctttctatt atttaggtgt tgcgacgcac     540
tactttacag atgctactca gccaatgcat gctgctaatt ttacagctgt cgacaggagt     600
gctataaagt ttcatagtgc ttttgaagat tatgtgacga caattcagga acagtttaaa     660
gtatcagatg gagagggaaa atataattta gtaaattcta atgatccgaa acagtggatc     720
catgaaacag cgagactcgc aaaagtggaa atcgggaaca ttaccaatga tgtgattaaa     780
```

```
tctcactata ataaaggaaa caatgctctt tggcagcaag aagttatgcc agctgttcag      840
agaagtttag aacaagccca aagaaatacg gcgggattta ttcatttatg gtttaaaaca      900
tatgttggaa aaacagctgc tgaagatatt gaaaatacta tagtgaaaga ttctagggga      960
gaagcaatac aagagaataa aaaatacttt gtagtaccaa gtgagttttt aaatagaggc     1020
ttaacatttg aagtgtatgc tgcttatgac tatgcgttat tatctaacca tgtggatgat     1080
aataatattc atggtacacc ggttcaaatt gtatttgata aagaaaataa tgggatcctt     1140
catcaaggag aaagtgcatt gttaaagatg acacaatcca actacgataa ttatgtattt     1200
ctaaattatt ctatcataac aaattgggta catcttgcaa aaagagaaaa caatactgca     1260
cagtttaaag tgtatccaaa tccaaataat ccaactgaat atttcatata tacagatggc     1320
tatccagtta attatcaaga aaaaggtaaa gagaaaagct ggattgtttt aggaaagaaa     1380
acggataaac caaaagcatg gaaatttata caggcggaat aa                        1422
```

<210> 98
<211> 473
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(25)

<400> 98

```
Met Lys Arg Lys Leu Cys Thr Trp Ala Leu Val Thr Ala Ile Ala Ser
 1               5                  10                  15
Ser Thr Ala Val Ile Pro Thr Ala Ala Glu Ala Cys Gly Leu Gly Glu
            20                  25                  30
Val Ile Lys Gln Glu Asn Gln Glu His Lys Arg Val Lys Arg Trp Ser
        35                  40                  45
Ala Glu His Pro His His Ser His Glu Ser Thr His Leu Trp Ile Ala
    50                  55                  60
Gln Asn Ala Ile Gln Ile Met Ser Arg Asn Gln Asp Lys Thr Val Gln
65                  70                  75                  80
Glu Asn Glu Leu Gln Phe Leu Asn Thr Pro Glu Tyr Lys Glu Leu Phe
                85                  90                  95
Glu Arg Gly Leu Tyr Asp Ala Asp Tyr Leu Asp Glu Phe Asn Asp Gly
            100                 105                 110
Gly Thr Gly Ile Ile Gly Ile Asp Gly Leu Ile Arg Gly Gly Trp Lys
        115                 120                 125
Ser His Phe Tyr Asp Pro Asp Thr Arg Lys Asn Tyr Lys Gly Glu Glu
    130                 135                 140
Glu Pro Thr Ala Leu Ser Gln Gly Asp Lys Tyr Phe Lys Leu Ala Gly
145                 150                 155                 160
Glu Tyr Phe Lys Lys Asn Asp Trp Lys Gln Ala Phe Tyr Tyr Leu Gly
                165                 170                 175
Val Ala Thr His Tyr Phe Thr Asp Ala Thr Gln Pro Met His Ala Ala
            180                 185                 190
Asn Phe Thr Ala Val Asp Arg Ser Ala Ile Lys Phe His Ser Ala Phe
        195                 200                 205
Glu Asp Tyr Val Thr Thr Ile Gln Glu Gln Phe Lys Val Ser Asp Gly
    210                 215                 220
Glu Gly Lys Tyr Asn Leu Val Asn Ser Asn Asp Pro Lys Gln Trp Ile
225                 230                 235                 240
His Glu Thr Ala Arg Leu Ala Lys Val Glu Ile Gly Asn Ile Thr Asn
                245                 250                 255
Asp Val Ile Lys Ser His Tyr Asn Lys Gly Asn Asn Ala Leu Trp Gln
```

```
        260                 265                 270
Gln Glu Val Met Pro Ala Val Gln Arg Ser Leu Glu Gln Ala Gln Arg
    275                 280                 285
Asn Thr Ala Gly Phe Ile His Leu Trp Phe Lys Thr Tyr Val Gly Lys
290                 295                 300
Thr Ala Ala Glu Asp Ile Glu Asn Thr Ile Val Lys Asp Ser Arg Gly
305                 310                 315                 320
Glu Ala Ile Gln Glu Asn Lys Lys Tyr Phe Val Val Pro Ser Glu Phe
                325                 330                 335
Leu Asn Arg Gly Leu Thr Phe Glu Val Tyr Ala Ala Tyr Asp Tyr Ala
            340                 345                 350
Leu Leu Ser Asn His Val Asp Asp Asn Asn Ile His Gly Thr Pro Val
        355                 360                 365
Gln Ile Val Phe Asp Lys Glu Asn Asn Gly Ile Leu His Gln Gly Glu
    370                 375                 380
Ser Ala Leu Leu Lys Met Thr Gln Ser Asn Tyr Asp Asn Tyr Val Phe
385                 390                 395                 400
Leu Asn Tyr Ser Ile Ile Thr Asn Trp Val His Leu Ala Lys Arg Glu
                405                 410                 415
Asn Asn Thr Ala Gln Phe Lys Val Tyr Pro Asn Pro Asn Asn Pro Thr
            420                 425                 430
Glu Tyr Phe Ile Tyr Thr Asp Gly Tyr Pro Val Asn Tyr Gln Glu Lys
        435                 440                 445
Gly Lys Glu Lys Ser Trp Ile Val Leu Gly Lys Lys Thr Asp Lys Pro
    450                 455                 460
Lys Ala Trp Lys Phe Ile Gln Ala Glu
465                 470
```

<210> 99
<211> 1053
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 99

```
atggcaaagc gttttattct ttcgatcgat ggtggtggca ttcgcgggat catcccggcg      60
gccatcctgg tggagctggc caagcggttg gaggggctgc cgcttcacaa ggcattcgac     120
atgatcgccg ggacatccac cggcggcatc attgcggcgg ggctgacatg cccgcatcct     180
gacgatgagg agacggcggc gtgcacgccg accgatcttc tcaagcttta tgtcgatcac     240
ggcggcaaga tcttcgagaa aaacccgatc ctcggcctca tcaacccatt cggcctcaac     300
gatccgcgct accagccaga tgagctggaa aacaggctga aggcgcagct cggcttgacg     360
gcgacgctcg ataaagggct caccaaggtg ctgatcacgg cctatgatat ccagcagcgg     420
caggcgctgt tcatggcaaa caccgacaac gagaacagca atttccgcta ctgggaggca     480
gcgcgggcga catcggccgc acccacctat tttccgccgg cgctgatcga aagggttggc     540
gagaagaaca aggacaagcg cttcgtgcca ttgatcgacg gcggcgtctt cgccaacgat     600
cctatccttg ccgcctatgt ggaggcgcga aagcagaaat ggggcaatga cgagctcgtt     660
ttcctgtcgc ttggtaccgg ccagcaaaac cgcccgatcg cctatcagga ggccaagggc     720
tggggcattt taggctggat gcagccgtct catgacacgc cgctgatctc gatcctgatg     780
cagggacagg cgagcaccgc ctcctatcag gccaatgcgc tgctcaatcc gcccggcacc     840
aagatcgact attcgaccgt ggtgacgaag gacaacgcgg cttcgctcag ctatttccgt     900
ctcgaccggc agctgagctc gaaggagaac gacgcgctgg acgacgcatc gcccgaaaac     960
atcagggcgc tgaaggcaat cgccgcgcaa atcatcaagg ataacgcgcc ggcgctcgac    1020
gaaatcgcca aacgcatcct ggccaaccaa taa                                 1053
```

<210> 100

<211> 350
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 100
```
Met Ala Lys Arg Phe Ile Leu Ser Ile Asp Gly Gly Gly Ile Arg Gly
 1               5                  10                  15
Ile Ile Pro Ala Ala Ile Leu Val Glu Leu Ala Lys Arg Leu Glu Gly
            20                  25                  30
Leu Pro Leu His Lys Ala Phe Asp Met Ile Ala Gly Thr Ser Thr Gly
        35                  40                  45
Gly Ile Ile Ala Ala Gly Leu Thr Cys Pro His Pro Asp Asp Glu Glu
    50                  55                  60
Thr Ala Ala Cys Thr Pro Thr Asp Leu Leu Lys Leu Tyr Val Asp His
65                  70                  75                  80
Gly Gly Lys Ile Phe Glu Lys Asn Pro Ile Leu Gly Leu Ile Asn Pro
                85                  90                  95
Phe Gly Leu Asn Asp Pro Arg Tyr Gln Pro Asp Glu Leu Glu Asn Arg
            100                 105                 110
Leu Lys Ala Gln Leu Gly Leu Thr Ala Thr Leu Asp Lys Gly Leu Thr
        115                 120                 125
Lys Val Leu Ile Thr Ala Tyr Asp Ile Gln Gln Arg Gln Ala Leu Phe
    130                 135                 140
Met Ala Asn Thr Asp Asn Glu Asn Ser Asn Phe Arg Tyr Trp Glu Ala
145                 150                 155                 160
Ala Arg Ala Thr Ser Ala Ala Pro Thr Tyr Phe Pro Pro Ala Leu Ile
                165                 170                 175
Glu Arg Val Gly Glu Lys Asn Lys Asp Lys Arg Phe Val Pro Leu Ile
            180                 185                 190
Asp Gly Gly Val Phe Ala Asn Asp Pro Ile Leu Ala Ala Tyr Val Glu
        195                 200                 205
Ala Arg Lys Gln Lys Trp Gly Asn Asp Glu Leu Val Phe Leu Ser Leu
    210                 215                 220
Gly Thr Gly Gln Gln Asn Arg Pro Ile Ala Tyr Gln Glu Ala Lys Gly
225                 230                 235                 240
Trp Gly Ile Leu Gly Trp Met Gln Pro Ser His Asp Thr Pro Leu Ile
                245                 250                 255
Ser Ile Leu Met Gln Gly Gln Ala Ser Thr Ala Ser Tyr Gln Ala Asn
            260                 265                 270
Ala Leu Leu Asn Pro Pro Gly Thr Lys Ile Asp Tyr Ser Thr Val Val
        275                 280                 285
Thr Lys Asp Asn Ala Ala Ser Leu Ser Tyr Phe Arg Leu Asp Arg Gln
    290                 295                 300
Leu Ser Ser Lys Glu Asn Asp Ala Leu Asp Asp Ala Ser Pro Glu Asn
305                 310                 315                 320
Ile Arg Ala Leu Lys Ala Ile Ala Ala Gln Ile Ile Lys Asp Asn Ala
                325                 330                 335
Pro Ala Leu Asp Glu Ile Ala Lys Arg Ile Leu Ala Asn Gln
            340                 345                 350
```

<210> 101
<211> 996
<212> DNA
<213> Bacteria

<400> 101

```
ttgtcgctcg tcgcgtcgct ccgccgcgcc cccggcgccg ccctggccct cgcgcttgcc      60
gccgccaccc tggccgtgac cgcgcagggc gcgaccgccg cccccgccgc ggccgccgcc     120
gaggccccgc ggctcaaggt gctcacgtac aacacgttcc tgttctcgaa gacgctctac     180
ccgaactggg gccaggacca ccgggccaag gcgatcccca ccgccccctt ctaccagggc     240
caggacgtcg tggtcctcca ggaggccttc gacaactccg cgtcggacgc cctcaaggcg     300
aactccgccg gccagtaccc ctaccagacc cccgtcgtgg gccgcggcac cggcggctgg     360
gacgccaccg gcgggtccta ctcctcgacc acccccgagg acggcggcgt gacgatcctc     420
agcaagtggc cgatcgtccg caaggagcag tacgtctaca aggacgcgtg cggcgccgac     480
tggtggtcca acaagggctt cgcctacgtc gtgctcaacg tgaacggcag caaggtgcac     540
gtcctcggca cccacgccca gtccaccgac ccgggctgct cggcgggcga ggcggtgcag     600
atgcggagcc gccagttcaa ggcgatcgac gccttcctcg acgccaagaa catcccggcg     660
ggcgagcagg tgatcgtcgc cggcgacatg aacgtcgact cgcgcacgcc cgagtacggc     720
accatgctcg ccgacgccgg tctggcggcg gccgacgcgc gcaccggcca cccgtactcc     780
ttcgacaccg agctgaactc gatcgcctcc gagcgctacc cggacgaccc gcgcgaggac     840
ctcgattacg tcctctaccg cgccgggaac gcccgccccg ccaactggac caacaacgtg     900
gtcctggaga agagcgcccc gtggaccgtc tccagctggg gcaagagcta cacctacacc     960
aacctctccg accactaccc ggtcaccggc ttctga                               996
```

<210> 102
<211> 331
<212> PRT
<213> Bacteria

<220>
<221> SIGNAL
<222> (1)...(39)

<400> 102

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Leu Ser Leu Val Ala Ser Leu Arg Arg Ala Pro Gly Ala Ala Leu Ala
 1               5                  10                  15
Leu Ala Leu Ala Ala Ala Thr Leu Ala Val Thr Ala Gln Gly Ala Thr
            20                  25                  30
Ala Ala Pro Ala Ala Ala Ala Ala Glu Ala Pro Arg Leu Lys Val Leu
        35                  40                  45
Thr Tyr Asn Thr Phe Leu Phe Ser Lys Thr Leu Tyr Pro Asn Trp Gly
    50                  55                  60
Gln Asp His Arg Ala Lys Ala Ile Pro Thr Ala Pro Phe Tyr Gln Gly
65                  70                  75                  80
Gln Asp Val Val Val Leu Gln Glu Ala Phe Asp Asn Ser Ala Ser Asp
                85                  90                  95
Ala Leu Lys Ala Asn Ser Ala Gly Gln Tyr Pro Tyr Gln Thr Pro Val
            100                 105                 110
Val Gly Arg Gly Thr Gly Gly Trp Asp Ala Thr Gly Gly Ser Tyr Ser
        115                 120                 125
Ser Thr Thr Pro Glu Asp Gly Gly Val Thr Ile Leu Ser Lys Trp Pro
    130                 135                 140
Ile Val Arg Lys Glu Gln Tyr Val Tyr Lys Asp Ala Cys Gly Ala Asp
145                 150                 155                 160
Trp Trp Ser Asn Lys Gly Phe Ala Tyr Val Val Leu Asn Val Asn Gly
                165                 170                 175
Ser Lys Val His Val Leu Gly Thr His Ala Gln Ser Thr Asp Pro Gly
            180                 185                 190
Cys Ser Ala Gly Glu Ala Val Gln Met Arg Ser Arg Gln Phe Lys Ala
        195                 200                 205
Ile Asp Ala Phe Leu Asp Ala Lys Asn Ile Pro Ala Gly Glu Gln Val
```

210 215 220
Ile Val Ala Gly Asp Met Asn Val Asp Ser Arg Thr Pro Glu Tyr Gly
225 230 235 240
Thr Met Leu Ala Asp Ala Gly Leu Ala Ala Ala Asp Ala Arg Thr Gly
245 250 255
His Pro Tyr Ser Phe Asp Thr Glu Leu Asn Ser Ile Ala Ser Glu Arg
260 265 270
Tyr Pro Asp Asp Pro Arg Glu Asp Leu Asp Tyr Val Leu Tyr Arg Ala
275 280 285
Gly Asn Ala Arg Pro Ala Asn Trp Thr Asn Asn Val Val Leu Glu Lys
290 295 300
Ser Ala Pro Trp Thr Val Ser Ser Trp Gly Lys Ser Tyr Thr Tyr Thr
305 310 315 320
Asn Leu Ser Asp His Tyr Pro Val Thr Gly Phe
325 330

<210> 103
<211> 2205
<212> DNA
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 103
atgagcgaga agaaggagat tcgcgttgcg ttgatcatgg ggggtggcgt cagcctcggc 60
agtttttcgg gtggtgcgct tctcaagacc atcgagctgc tgcagcacac tgcccgcggt 120
ccggcgaaga tcgatgtcgt gaccggtgcc tcggcgggaa gcatgacgct gggcgtagtc 180
atctaccacc tcatgcgggg atcgtcgacc gatgagattc tccgcgatct gaggcggtcg 240
tgggtggaaa tgatctcgtt cgacggcctc tgtccgccga acctgtcccg tcacgacaag 300
ccgagcctgt tttccgatga gatcgtccgg aagatcgcgg ccaccgtcat cgatatgggg 360
cgcaagctcg aggcggctcc tcatccgctt ttcgccgacg aactcgtagc ctcgttcgca 420
ctgacgaacc tgaacggcat ccccgcccgt acggagggcc agctcatccg gcaggcaaag 480
ggaggcggag ggtccgagaa gggctcgaaa tccgttttcg ccgacgccgt gcagactacc 540
tttcaccacg acgtgatgcg attcgtggtg cggcgcgatc acaacgggca aggcagcctg 600
ttcgacagcc gttaccgggc acgcatactc cctccatgga atgttgggaa gggcggcgat 660
gcatgggaag cctttcgcac ggcggctgtt gcctcggggg cgtttccggc cgcatttcct 720
cccgtcgaga tcagccgcaa ccgcgacgaa ttcaacatct ggcccgatcg catcgaggac 780
cagaaggcat ttacgttcga ttacgtggac ggcggggtac ttcgcaacga acccctccgg 840
gaggcgattc acctggccgc gctgcgcgat gagggagcga cggacatcga gcgtgtgttc 900
atcctcatcg acccgaacat cagcggcacc ggcgaggtct tcccgctctc ctataaccag 960
cagatgcgga tcaagccgaa ctacgattcc aacggcgacg tccgacagta cgatctcgat 1020
gtgccggact acaccggcaa tctgatcggg gcgatcggtc ggctgggttc ggtgatcgtc 1080
gggcaggcga cgttccgcga ctggctcaag gctgccaaag tgaacagcca gatcgagtgg 1140
cgacgggaat tgctgcccat tctccgcgac ctgaacccga accccgggga ggaggcgcgc 1200
aggggcgtga acgggatgat cgacaagatc taccggcaaa agtatcagcg cgccctcgag 1260
tcaaagagcg ttccggtcga ggaggtggaa cggcgcgttg ccgaagacat cgaacgggac 1320
ctggcgcggc gccgttcgga ggccggcgac aacgacttca ttgcccggct cctcctgctc 1380
gtcgacctga tcggcaacct gcgtgagaag cagaagctga acatggtggc gatcaccccc 1440
gcttccgcgc cgcacaacga cgggcgcccc ttgccgctgg ccggcaattt tatgttcagc 1500
ttcggggggt tcttcaggga ggagtacagg caatacgact tctcggtcgg cgaattcgca 1560
gcatggaacg tcctgagcac gccggcctcc gagacgccct ttcttgccga gaccgccccg 1620
aaaccgcccg cccgacctcc ccagccgccg gcaatcaatc ctacctaccg ctcactcggc 1680
ccgcccatcc agcagcggtt cgaggagttc gttcgtgggc acgttcgcgc ctttatcgct 1740
tcggtcgctc cgctgggaac gagagggatc gtcacgggca agattggcgg aaagcttcga 1800
acgatgctga tggcctcgcg caacgggaaa tcagagtact tccggcttcg cctctccggc 1860
gttgacgggc tctacctccg aggctccaag ggccgcaacc tgagggcggt taacggatcg 1920

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atcgacacgg tcgtcggcgt ctatatcgac gaggaagatc agcaccgcga tgagtttttc     1980
ggtccccatg tcttcggcgc gaacggctca ggctttacga tggaactatg ggagtcccgc     2040
ggtttttttcg ggcgtgatcg tcgcgtcgct gtgatcgagt tggagaacaa ccccggcggg    2100
ttcgcaatcg ccgccggatg caggcggcgg cccggcgtgg tgctggatat ggccaggcgt     2160
aacgggcagc cactgcggac ggtggatgtg atggaatttg cgtga                     2205

<210> 104
<211> 734
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<400> 104
Met Ser Glu Lys Lys Glu Ile Arg Val Ala Leu Ile Met Gly Gly Gly
1               5                   10                  15
Val Ser Leu Gly Ser Phe Ser Gly Gly Ala Leu Leu Lys Thr Ile Glu
            20                  25                  30
Leu Leu Gln His Thr Ala Arg Gly Pro Ala Lys Ile Asp Val Val Thr
        35                  40                  45
Gly Ala Ser Ala Gly Ser Met Thr Leu Gly Val Val Ile Tyr His Leu
    50                  55                  60
Met Arg Gly Ser Ser Thr Asp Glu Ile Leu Arg Asp Leu Arg Arg Ser
65                  70                  75                  80
Trp Val Glu Met Ile Ser Phe Asp Gly Leu Cys Pro Pro Asn Leu Ser
                85                  90                  95
Arg His Asp Lys Pro Ser Leu Phe Ser Asp Glu Ile Val Arg Lys Ile
            100                 105                 110
Ala Ala Thr Val Ile Asp Met Gly Arg Lys Leu Glu Ala Ala Pro His
        115                 120                 125
Pro Leu Phe Ala Asp Glu Leu Val Ala Ser Phe Ala Leu Thr Asn Leu
    130                 135                 140
Asn Gly Ile Pro Ala Arg Thr Glu Gly Gln Leu Ile Arg Gln Ala Lys
145                 150                 155                 160
Gly Gly Gly Gly Ser Glu Lys Gly Ser Lys Ser Val Phe Ala Asp Ala
                165                 170                 175
Val Gln Thr Thr Phe His His Asp Val Met Arg Phe Val Val Arg Arg
            180                 185                 190
Asp His Asn Gly Gln Gly Ser Leu Phe Asp Ser Arg Tyr Arg Ala Arg
        195                 200                 205
Ile Leu Pro Pro Trp Asn Val Gly Lys Gly Gly Asp Ala Trp Glu Ala
    210                 215                 220
Phe Arg Thr Ala Ala Val Ala Ser Gly Ala Phe Pro Ala Ala Phe Pro
225                 230                 235                 240
Pro Val Glu Ile Ser Arg Asn Arg Asp Glu Phe Asn Ile Trp Pro Asp
                245                 250                 255
Arg Ile Glu Asp Gln Lys Ala Phe Thr Phe Asp Tyr Val Asp Gly Gly
            260                 265                 270
Val Leu Arg Asn Glu Pro Leu Arg Glu Ala Ile His Leu Ala Ala Leu
        275                 280                 285
Arg Asp Glu Gly Ala Thr Asp Ile Glu Arg Val Phe Ile Leu Ile Asp
    290                 295                 300
Pro Asn Ile Ser Gly Thr Gly Glu Val Phe Pro Leu Ser Tyr Asn Gln
305                 310                 315                 320
Gln Met Arg Ile Lys Pro Asn Tyr Asp Ser Asn Gly Asp Val Arg Gln
                325                 330                 335
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Tyr Asp Leu Asp Val Pro Asp Tyr Thr Gly Asn Leu Ile Gly Ala Ile
            340                 345                 350
Gly Arg Leu Gly Ser Val Ile Val Gly Gln Ala Thr Phe Arg Asp Trp
        355                 360                 365
Leu Lys Ala Ala Lys Val Asn Ser Gln Ile Glu Trp Arg Arg Glu Leu
    370                 375                 380
Leu Pro Ile Leu Arg Asp Leu Asn Pro Asn Pro Gly Glu Glu Ala Arg
385                 390                 395                 400
Arg Gly Val Asn Gly Met Ile Asp Lys Ile Tyr Arg Gln Lys Tyr Gln
                405                 410                 415
Arg Ala Leu Glu Ser Lys Ser Val Pro Val Glu Glu Val Glu Arg Arg
            420                 425                 430
Val Ala Glu Asp Ile Glu Arg Asp Leu Ala Arg Arg Arg Ser Glu Ala
        435                 440                 445
Gly Asp Asn Asp Phe Ile Ala Arg Leu Leu Leu Leu Val Asp Leu Ile
    450                 455                 460
Gly Asn Leu Arg Glu Lys Gln Lys Leu Asn Met Val Ala Ile Thr Pro
465                 470                 475                 480
Ala Ser Ala Pro His Asn Asp Gly Arg Pro Leu Pro Leu Ala Gly Asn
                485                 490                 495
Phe Met Phe Ser Phe Gly Gly Phe Phe Arg Glu Glu Tyr Arg Gln Tyr
            500                 505                 510
Asp Phe Ser Val Gly Glu Phe Ala Ala Trp Asn Val Leu Ser Thr Pro
        515                 520                 525
Ala Ser Glu Thr Pro Phe Leu Ala Glu Thr Ala Pro Lys Pro Pro Ala
    530                 535                 540
Arg Pro Pro Gln Pro Pro Ala Ile Asn Pro Thr Tyr Arg Ser Leu Gly
545                 550                 555                 560
Pro Pro Ile Gln Gln Arg Phe Glu Glu Phe Val Arg Gly His Val Arg
                565                 570                 575
Ala Phe Ile Ala Ser Val Ala Pro Leu Gly Thr Arg Gly Ile Val Thr
            580                 585                 590
Gly Lys Ile Gly Gly Lys Leu Arg Thr Met Leu Met Ala Ser Arg Asn
        595                 600                 605
Gly Lys Ser Glu Tyr Phe Arg Leu Arg Leu Ser Gly Val Asp Gly Leu
    610                 615                 620
Tyr Leu Arg Gly Ser Lys Gly Arg Asn Leu Arg Ala Val Asn Gly Ser
625                 630                 635                 640
Ile Asp Thr Val Val Gly Val Tyr Ile Asp Glu Glu Asp Gln His Arg
                645                 650                 655
Asp Glu Phe Phe Gly Pro His Val Phe Gly Ala Asn Gly Ser Gly Phe
            660                 665                 670
Thr Met Glu Leu Trp Glu Ser Arg Gly Phe Phe Gly Arg Asp Arg Arg
        675                 680                 685
Val Ala Val Ile Glu Leu Glu Asn Asn Pro Gly Gly Phe Ala Ile Ala
    690                 695                 700
Ala Gly Cys Arg Arg Arg Pro Gly Val Val Leu Asp Met Ala Arg Arg
705                 710                 715                 720
Asn Gly Gln Pro Leu Arg Thr Val Asp Val Met Glu Phe Ala
                725                 730
```

<210> 105
<211> 756
<212> DNA
<213> Unknown

<220>

<223> Obtained from an environmental sample.

<400> 105
atgaaccgtt gtcggaactc actcaacctc caacttcgcg cggtgaccgt ggcggcgttg 60
gtagtcgtcg catcctcggc cgcgctggcg tgggacagcg cctcgcgcaa tccgacccat 120
cccacccaca gctacctcac cgaatacgcc atcgatcagc ttggggtggc gcggccggag 180
ctccggcaat accgcaagca gatcatcgag ggcgccaaca ccgagctgca cgaactgcca 240
gtcaagggga cggcctatgg cctcgacctc gacgccaagc ggcgggaaca ccgcggcacc 300
aatgccggga cagacgacat cgccggctgg tgggcggaaa gcctccaagc ctatcgcgcc 360
ggtgccaagg aacgcgccta cttcgtgctg gggtggtgc tgcacatggt cgaggacatg 420
ggcgtgccgg cgcacgcgaa cggcgtctac caccagggca acctgactga attcgacaat 480
ttcgagttca tgggactgtc gaactggaag ccctctttcg ccgacatcaa ccggaccgat 540
ccgggctacg ccgacccgtc gcgctactac gagttcagcc gagattggac ggcggcagac 600
gcacccggct atcgcgaccg cgacagcttc tcgaagacct gggttctcgc cagcccggcc 660
gaacgtcagc tgcttcagaa ccgccagggc cggaccgcca cggtcgccat gtgggcgtta 720
cggagcgcga cgaaggcgtt cgccgggaaa ccctag 756

<210> 106
<211> 251
<212> PRT
<213> Unknown

<220>
<223> Obtained from an environmental sample.

<221> SIGNAL
<222> (1)...(30)

<400> 106
Met Asn Arg Cys Arg Asn Ser Leu Asn Leu Gln Leu Arg Ala Val Thr
1 5 10 15
Val Ala Ala Leu Val Val Val Ala Ser Ser Ala Ala Leu Ala Trp Asp
20 25 30
Ser Ala Ser Arg Asn Pro Thr His Pro Thr His Ser Tyr Leu Thr Glu
35 40 45
Tyr Ala Ile Asp Gln Leu Gly Val Ala Arg Pro Glu Leu Arg Gln Tyr
50 55 60
Arg Lys Gln Ile Ile Glu Gly Ala Asn Thr Glu Leu His Glu Leu Pro
65 70 75 80
Val Lys Gly Thr Ala Tyr Gly Leu Asp Leu Asp Ala Lys Arg Arg Glu
85 90 95
His Arg Gly Thr Asn Ala Gly Thr Asp Asp Ile Ala Gly Trp Trp Ala
100 105 110
Glu Ser Leu Gln Ala Tyr Arg Ala Gly Ala Lys Glu Arg Ala Tyr Phe
115 120 125
Val Leu Gly Val Val Leu His Met Val Glu Asp Met Gly Val Pro Ala
130 135 140
His Ala Asn Gly Val Tyr His Gln Gly Asn Leu Thr Glu Phe Asp Asn
145 150 155 160
Phe Glu Phe Met Gly Leu Ser Asn Trp Lys Pro Ser Phe Ala Asp Ile
165 170 175
Asn Arg Thr Asp Pro Gly Tyr Ala Asp Pro Ser Arg Tyr Tyr Glu Phe
180 185 190
Ser Arg Asp Trp Thr Ala Ala Asp Ala Pro Gly Tyr Arg Asp Arg Asp
195 200 205
Ser Phe Ser Lys Thr Trp Val Leu Ala Ser Pro Ala Glu Arg Gln Leu
210 215 220

```
Leu Gln Asn Arg Gln Gl   rg Thr Ala Thr Val Ala Met Trp Ala Leu
225                 230                 235                 240
Arg Ser Ala Thr Lys Ala  he Ala Gly Lys Pro
                245                 250
```